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THE DISCHARGE OF IMPULSES FROM GANGLION CELLS

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UNDER normal physiological conditions the discharge of impulses from sympathetic ganglion cells appears to occur only when these cells are bombarded by impulses in preganglionic fibres [cf. Govaerts, 1935, 1936]. When an impulse in a preganglionic fibre reaches that part which establishes functional connexion (the synapse) with a ganglion cell, it acts on that cell, and may, after a brief interval (the synaptic delay), cause it to discharge an impulse along its axon (the postganglionic fibre). This paper gives an account of investigations into this action of the preganglionic impulse on the ganglion cell. A preliminary account of some of these investigations has already been given [Eccles, 1936*a*]. The methods employed in recording electrically from the ganglion [Eccles, 1935*a*, 1936*b*] and mechanically from the nictitating membrane [Eccles & Magladery, 1937*a*] have already been fully described.

A. THE INTERACTION BETWEEN AN ANTIDROMIC AND A PREGANGLIONIC VOLLEY

In 1934 Brown studied this interaction for the S_1 ganglion cells which innervate the nictitating membrane. Single stimuli, adequate to set up maximal volleys, were applied to the preganglionic and postganglionic trunks of the superior cervical ganglion, and the resulting contractions of the nictitating membrane were recorded with an optical isometric myograph. When the preganglionic volley was set up between 9 and 5 msec. before the antidromic volley, the contraction of the nictitating membrane was no larger than for the antidromic volley alone, i.e. under such conditions the refractory period of the ganglion cells or postganglionic fibres

allowed only a single impulse to traverse every postganglionic fibre. With longer or shorter intervals, the increased contraction showed that there was more than one impulse in some of the postganglionic fibres, i.e. the preganglionic volley caused some ganglion cells to discharge impulses at such a long time earlier or later than the antidromic volley that blocking by refractory period no longer occurred.

In Fig. 1 the conditions of Brown's experiments are plotted, as explained in the legend, on time-distance co-ordinates [cf. Eccles & Sherrington, 1931, p. 520], using conduction velocities of 17 and 6 metres a second for the pre- and postganglionic fibres respectively

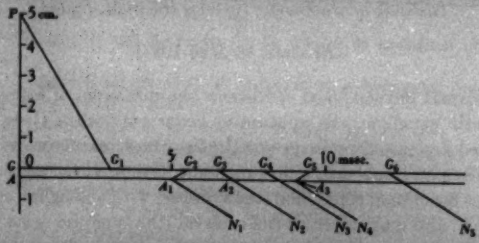

Fig. 1. The courses of impulses in the pre- and postganglionic pathways of the superior cervical ganglion are plotted on time (abscissa) and distance co-ordinates, P, G and A being respectively the positions of the preganglionic electrodes, the ganglion, and the electrodes at which antidromic volleys are set up in the postganglionic trunk. The oblique lines represent the passage of impulses in the pre- and postganglionic fibres, the conduction velocities being assumed to be 17 and 6 metres per sec. respectively. An approximation is introduced by neglecting the length of the ganglion, but this does not significantly affect the argument developed in the text.

[cf. Eccles, 1935*a*]. PG_1 shows the time course of the fastest preganglionic impulses of a maximal preganglionic volley. This volley sets up the discharge of impulses along the postganglionic fibres and a consequent absolutely refractory period, from which recovery is first detectable at A_2 (9 msec. after P) when a testing stimulus is applied through electrodes placed at A on the postganglionic trunk. Since the absolutely refractory period of the postganglionic fibres is about 2 msec. [Brown, 1934; Eccles, 1936*b*], the earliest postganglionic impulses to be discharged in response to PG_1 must pass approximately through A_2 and so be represented by $G_2A_2N_2$. The shortest synaptic delay is thus G_1G_2, its duration 3·5 msec., being in close agreement with the value derived from direct electrical measurements [Eccles, 1935*a*].

Now, when the maximal antidromic volley is set up earlier than A_1, i.e. less than 5 msec. after P, the response of the nictitating membrane shows that PG_1 has set up a discharge of impulses from some ganglion cells, which of course must occur after recovery from the refractory period following the antidromic volley. As the antidromic volley is moved later, i.e. nearer to A_1, PG_1 sets up a discharge from progressively fewer ganglion cells, until, with an antidromic volley A_1G_2, limiting conditions are reached, any further retardation preventing the discharge from all ganglion cells. It appears from Fig. 1 that, under such limiting conditions, the antidromic volley reaches the ganglion cells even before the earliest discharge $G_2A_2N_2$ would be set up by PG_1, and certainly considerably before more delayed discharges. Moreover, it has been shown that the antidromic volley actually penetrates as far as the locus where the ganglion cell discharge arises and there gives rise to a refractory period [Eccles, 1936*b*, p. 11]. The interval after the limiting antidromic volley, A_1G_2, at which PG_1 must set up its minimal discharge, would be longer than the true absolutely refractory period of the ganglion cells (*ca.* 2 msec. [Eccles, 1936*b*]). Also, presumably, it would be shorter than their functional refractory period as determined by two maximal preganglionic volleys (*ca.* 3 msec. [Eccles, 1935*a*]), for under these latter conditions the second preganglionic volley is diminished in size by the preceding volley. Thus a value of about 2·5 msec. after G_2, as represented by the point G_4, may be taken as sufficiently accurate to illustrate the argument, G_4N_3 thus representing the minimal postganglionic discharge which PG_1 produces after the antidromic volley A_1G_2. Further, PG_1 is unable to set up a discharge later than G_4, for, as we have seen, any further retardation of the antidromic volley beyond A_1G_2 prevents all discharge in response to PG_1. Hence G_1G_4, 5 msec. in duration, represents the upper limiting value to the synaptic delay. Presumably the ganglion cells discharging with that long synaptic delay are likely to be amongst those cells which normally respond during the latter part of the temporally dispersed ganglionic discharge, hence the maximum lengthening of synaptic delay is probably less than 1·5 msec. which is the difference between the above values for the longest and shortest synaptic delays.

The conclusions of this argument are not essentially affected by variations in the choice of the conduction times, refractory periods, etc., so long as these variations are kept within probable limits. Action potential records [Eccles, 1936*b*, pp. 9–13] indicate that an antidromic volley acts similarly on the discharge of impulses which a preganglionic volley sets up from S_2 ganglion cells, the maximum lengthening of synaptic

delay being probably not greater than 2·5 msec. *It may, therefore, be concluded that a preganglionic volley has lost its power of setting up a discharge of impulses from S_1 and S_2 ganglion cells, if that discharge is delayed by more than 1·5 and 2·5 msec. respectively by the refractory period following an antidromic volley.*

In most experiments Brown [1934] found that, as described above, a single antidromic volley, when suitably timed, blocked all the discharge which a single preganglionic volley sets up from ganglion cells, and hence concluded that a preganglionic volley sets up the discharge of no more than a single impulse from each ganglion cell [cf. Eccles & Sherrington, 1931, pp. 519–23]. Since an impulse discharged from a ganglion cell gives rise to a refractory period of that cell identical in all respects with that following an antidromic impulse [Eccles, 1935*a*, 1936*b*], the absence of a second discharge parallels the prevention of all discharge by a suitably timed antidromic volley, and again demonstrates the short duration of the excitatory effect produced by a preganglionic volley acting on the ganglion cells.

However, in a few experiments Brown found that an antidromic volley always failed to block completely the discharge set up by a single preganglionic volley, and in explanation he suggested that the discharge of single impulses was so asynchronous that a single antidromic volley was always ineffective in blocking some impulses. In Fig. 1 we have seen that A_2G_5 was the earliest time at which an antidromic volley could be set up after the earliest ganglionic discharge, $G_2A_2N_2$. According to Brown's suggestion some ganglion cells are capable of discharging impulses in response to PG_1 *after* the refractory period set up by A_2G_5, i.e. after G_6. The synaptic delay of such a discharge would be greater than 9 msec., hence a temporal dispersion of more than 5·5 msec. would have to occur. The only possible alternative explanation is that a preganglionic volley sets up the discharge of more than one impulse from some ganglion cells. However, in the present attempt to repeat these experiments, it was found that, only when it was not maximal, did an antidromic volley fail to block all the discharge evoked by a single preganglionic volley; hence there appears to be no necessity to postulate that, in some experiments, a single preganglionic volley sets up either a repetitive discharge from some ganglion cells or a discharge of single impulses having a large temporal dispersion.

Thus it may be concluded that, in so far as the setting up of a discharge of impulses is concerned, a single preganglionic volley exerts on ganglion cells an excitatory action which increases and decays so rapidly that only for a

very brief period (e.g. within extreme limits of 2·2–5 msec. for S_1 ganglion cells) is it above the liminal intensity at which a discharge is set up. The discharge of an impulse is a type of explosive act of a ganglion cell, and the brief action of preganglionic impulses in setting up this discharge resembles that of a detonator; hence it will be called the "detonator action".

B. The relationship of central excitatory state to the discharge of impulses by ganglion cells

It has already been shown that a single preganglionic volley produces in ganglion cells an increased excitability which decays gradually over a period as long as 200 msec. [Eccles, 1935*b*], and which has been called the central excitatory state (C.E.S.) on analogy with the similar prolonged excitatory condition exhibited by motoneurones of the spinal cord. Further, this excitatory state has been shown to be closely associated with a negativity (the *N* wave) of the soma of the ganglion cell relative to its axon. This *N* wave of the ganglion cell does not begin until about the end of the synaptic delay and does not reach a maximum until about 15–20 msec. after the arrival of a preganglionic volley at the synapses of these cells [Eccles, 1935*c*, pp. 473, 478; Eccles, 1936*b*, p. 9]. A similar late maximum (at about 10–15 msec.) for the C.E.S. set up by a preganglionic volley was suggested by experiments in which the excitability was tested by a second preganglionic volley, both volleys being set up by similar stimuli applied through the same electrodes. It was originally pointed out [Eccles, 1935*b*, p. 209] that such a late maximum of the facilitated response may be due to a diminution in size of the second preganglionic volley, for as long as 15 msec., by the refractory period following the first preganglionic volley. However, the similar late maximum for the closely associated *N* wave suggests that the C.E.S. which a preganglionic volley sets up in the ganglion cells also develops to a late maximum, and possibly also does not begin until about the end of the synaptic delay. This time course is confirmed by the experiments described in section C.

This late development establishes that the C.E.S. produced by a single preganglionic volley plays no significant part in relation to the detonator action of that volley, for this latter has set up the discharge of impulses before any appreciable C.E.S. has been produced. Moreover, by the time that the C.E.S. has attained its maximum intensity, there will have been almost complete recovery of the ganglion cells from the refractory period following the initial discharge, and yet there is no further discharge of impulses. A similar absence of discharge occurs during the summed C.E.S. set up by two or more volleys in quick succession. *Hence it may be concluded*

that ganglion cells are not excited to discharge impulses even by the largest intensities of C.E.S. *that normally can be produced by preganglionic impulses.*

The action of nicotine provides further evidence dissociating C.E.S. from the detonator action, for in small doses it greatly diminishes C.E.S., and at the same time even makes it easier for preganglionic impulses to set up the discharge of impulses from ganglion cells [Eccles, 1935*b*].

Thus the sole action of C.E.S. *appears to be a lowering of the threshold at which the detonator action of preganglionic impulses sets up a discharge from ganglion cells, i.e. it may be regarded as increasing the explosiveness of the ganglion cells.* The C.E.S. produced by a preganglionic volley is developed too late to have any significant action in lowering the detonator threshold for that volley. It must, however, be remembered that repetitive discharge along preganglionic fibres is usual under physiological conditions [Adrian *et al.* 1931], the C.E.S. set up by any impulse being thus normally of importance in lowering the detonator threshold for later impulses.

C. The detonator action of preganglionic impulses

In the first section it was shown that a preganglionic volley sets up a discharge of impulses from ganglion cells by means of a brief excitatory action (the detonator action) which it exerts on them. Now it has been shown [Eccles, 1935*b*, p. 216] that in most experiments a single impulse in a single preganglionic fibre fails to set up a discharge from any ganglion cells, i.e. its detonator action is subliminal. A liminal intensity is attained only when the ganglion cell is bombarded by more than one impulse of a single preganglionic volley. There must, therefore, be a summation of the detonator actions exerted by impulses reaching different synapses on a ganglion cell, and this summation must occur during the brief period of the synaptic delay. This fact suggests the following method by which the detonator action may be more directly investigated.

Single volleys are set up in two separate groups of preganglionic fibres, and the consequent discharge of impulses from ganglion cells is determined for each stimulus interval by analysing the spike action potentials led from the postganglionic fibres. Any effect which the first volley might exert on the second is measured by subtracting the action potential set up by the first volley from that set up by the combined volleys, the subtracted action potential being then compared with the action potential evoked by the second volley alone.

Fig. 2 shows a typical series of such action potentials (recorded from the postganglionic trunk of the superior cervical ganglion) from an experiment in which one volley is set up in one branch of the annulus Vieussens

and the other volley in the other branch. Both volleys are submaximal for the S_1 preganglionic fibres, the complex shape of the spike potential being due to the subsidiary S_1 waves which separate out on account of differences in the conduction velocities in different groups of the S_1 preganglionic fibres [cf. Eccles, 1935*a*, p. 191]. It is obvious that at all intervals except 4·5 msec. the first volley enables the second to set up a discharge from many ganglion cells in addition to those which respond to the second volley alone (spatial facilitation). These facilitated ganglion cells must be subliminally excited by each single volley, these two subliminal excitations, when summed, being adequate to set up a discharge. Preganglionic fibres from each branch of the annulus Vieussens must thus enter into synaptic relationship with such ganglion cells. The subtracted action potentials of Fig. 2 show that, when the volleys are simultaneous, this spatial facilitation is probably maximal, i.e. it is effective in producing a discharge from the largest number of ganglion cells; but this number is still large at an interval of 2·3 msec., while at 4·5 msec. effective summation occurs in very few, if any, ganglion cells. Temporal facilitation (shortening of synaptic delay) is also clearly present at intervals of 1·4 and 2·3 msec., but such facilitation is never large with simultaneous stimuli, and usually it progressively increases as the stimulus interval is lengthened to about 2 msec. At intervals longer than 4·5 msec. spatial and temporal facilitation again develop, the observation at 17·6 msec. interval (Fig. 2) showing practically maximal conditions. The time course of the decay of this second facilitation wave establishes its identity with the facilitation wave determined for two volleys in the same preganglionic fibres [Eccles, 1935*b*], i.e. it is due to the C.E.S. of the ganglion cells. The rising phase of the second facilitation wave thus gives the course of C.E.S. development when its determination is unaffected by

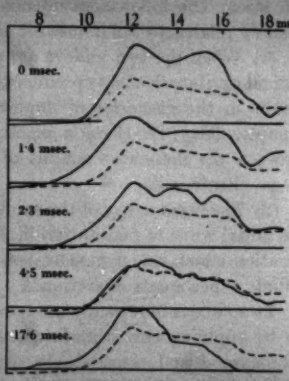

Fig. 2. Single submaximal stimuli have been applied to each of the branches of the annulus Vieussens at various intervals apart. The contanuous lines show the ganglionic action potentials (S_1 spike responses only) produced by the second stimulus at the indicated intervals, as determined by subtracting the first action potential from the combined action potential. The broken lines show the action potential set up by the second stimulus alone, all stimuli being synchronized at the zero of the time scale.

the refractoriness of preganglionic fibres, and it shows that, in this part of its time course also, the C.E.S. set up by a preganglionic volley resembles the *N* wave, having a delayed rise to a maximum at about 15 msec. (cf. section B). On the other hand, there must be a very rapid decay of the excitatory effect responsible for the facilitation at short intervals, for it has practically disappeared when tested at a stimulus interval of 4·5 msec. The following evidence shows that this excitatory effect is directly related to the detonator action of preganglionic impulses.

(1) When the two volleys are simultaneous, summation of the subliminal excitatory effects produced in many ganglion cells by each volley results in the discharge of impulses from these cells after the normal synaptic delay, i.e. there is exactly the type of summation that occurs between the detonator actions of the individual impulses of a single preganglionic volley.

(2) The rapid decay of the subliminal excitatory effect responsible for the initial wave of facilitation in Fig. 2 corresponds closely to the short duration which was demonstrated for the detonator action by the antidromic experiments of section A.

Two volleys in separate groups of preganglionic fibres may also be set up by applying two stimuli to the cervical sympathetic, the first being submaximal and considerably weaker than the second [cf. Lorente de Nó, 1935*a*, *b*]. So long as the second volley is set up during the refractory period of the first volley, these volleys must traverse separate groups of preganglionic fibres. The disturbing effects of post-kathodal depression have been avoided by applying the stimuli through separate pairs of electrodes on the cervical sympathetic. Further, if the weaker stimulus is applied near the ganglion and the stronger to the cervical sympathetic at the root of the neck, the range of intervals over which the ganglion cells will be subjected to bombardment by two volleys in entirely separate groups of preganglionic fibres is extended by twice the conduction time between the two sets of electrodes. This procedure has, therefore, been adopted in most of the present experiments, and Fig. 3 shows a series of subtracted action potentials (determined as for Fig. 2) for the various stimulus intervals. On account of its longer preganglionic conduction time, the larger volley set up distally from the ganglion will reach the ganglion slightly later than the smaller volley even in the first observation of Fig. 3. There the subtracted curve shows an early spike considerably above the control response to the second volley alone. This early spike must have arisen either on account of a shortening of the synaptic delay of some ganglion cells which also respond to the second

volley alone (temporal facilitation), or on account of the response of additional ganglion cells (spatial facilitation), and is probably due to both these types of facilitation. The smaller spike with an interval of

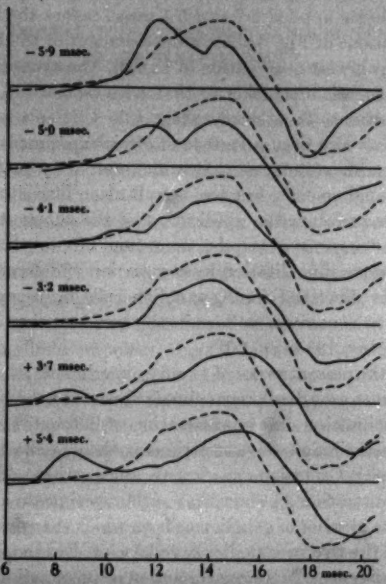

Fig. 3. A stimulus has been applied to the cervical sympathetic near the superior cervical ganglion at the indicated times after (observations 1, 2, 3 and 4) and before (observations 5 and 6) the application of a stronger stimulus through electrodes placed on it at the root of the neck, i.e. about 11 cm. from the ganglion. Both stimuli were submaximal for *S*, preganglionic fibres, and the stimulus intervals were chosen so that the volley set up near the ganglion always reached the ganglion first. At each stimulus interval the action potential evoked by the second volley (shown by the continuous lines) has been determined as in Fig. 2 by subtracting the first action potential from the combined action potential. The broken lines show the action potentials set up by the second volley alone, all stimuli being synchronized at the zero of the time scale as in Fig. 2.

5 msec. indicates facilitation of fewer ganglion cells, but the synaptic delay is still further shortened, and comparison of the observation at 4·1 msec. interval with that at 3·2 msec. shows that in the former there is still a little very early facilitated response. Thus Fig. 3 corresponds

closely with Fig. 2, lengthening of the interval between the times of arrival of the two volleys being accompanied in both by a rapid disappearance of spatial facilitation and by an initial increase in temporal facilitation. As the volley interval is still further lengthened, the smaller stimulus now being applied 3·7 and 5·4 msec. before the larger, the two lowest observations of Fig. 3 show a development of the facilitation due to C.E.S. (cf. the lowest observation of Fig. 2). The synaptic delay of this facilitated response is much shorter than with facilitation by summation of detonator actions. To a small extent this difference is detectable in Fig. 2, and it has also been a feature of other experiments.

Similar experiments suggest that summation of detonator actions also occurs for S_2 ganglion cells, but the complication introduced by the preceding S_1 spike prevents the application of the subtraction method in determining the response set up by the second of two successive volleys. In addition, temporal facilitation by summation of detonator actions has previously been described for S_2 ganglion cells in experiments on the interaction of two maximal volleys in the two branches of the annulus Vieussens [Eccles, 1935*a*, p. 201].

In eight of the present series of twenty experiments, the interaction of volleys in separate groups of preganglionic fibres did not reveal any spatial or temporal facilitation due to summation of detonator actions, and in several of the remaining twelve experiments these facilitations were only slightly developed. Occlusion experiments, such as those already described [Eccles, 1935*a*, section J], show that such experiments are not entirely explicable by an absence of ganglion cells on which there is an overlapping distribution of the two preganglionic volleys [cf. Eccles, 1935*b*, p. 211]. However, the absence of a detonator action of preganglionic impulses on ganglion cells must not then be assumed on account of this failure to demonstrate it by summation, for a preganglionic volley sets up a discharge which the investigations described in section A show to be produced by a normal brief detonator action on the ganglion cells. Presumably, in any such ganglion cell, there is little or no summation of the detonator actions exerted at its various synapses, the discharge set up from the ganglion by a single preganglionic volley being due to the supraliminal detonator actions of individual preganglionic impulses. A similar explanation has already been offered for those experiments in which diminution of a preganglionic volley was not accompanied by the relative increase usually observed in the subliminal fringe [Eccles, 1935*b*, p. 216]. In such experiments the existence of a small subliminal fringe shows that single preganglionic impulses do not all exert supraliminal detonator

actions at the synapses. There is merely no appreciable summation of any subliminal detonator actions which may be exerted at different synapses on a ganglion cell.

D. The relation of the detonator response to the synaptic delay

It has already been seen that, when it occurs, summation of the subliminal detonator actions of preganglionic impulses takes place during the synaptic delay, i.e. within about 3 msec., for the S_1 ganglion cells. Such a time would be too short for diffusion to allow a summation of the quanta of any hypothetical chemical mediator liberated at the different synapses; hence the subliminal detonator actions exerted by each preganglionic impulse would have to sum actually within the ganglion cell. On the other hand, if synaptic transmission is mediated by the action currents of the preganglionic impulses (see section E), there would, of course, be an instantaneous summation of the exciting effects of the different preganglionic impulses, i.e. of their action currents, but such a direct summation would be so diminished on account of the distance between the different synapses that it could hardly be responsible for the observed summation of detonator actions; thus on this hypothesis also it seems necessary to invoke a summation of subliminal responses of the ganglion cell. The existence of such a response of a nerve cell is also indicated by the experiments of Lorente de Nó [1935*c*, 1936], who showed that with oculomotor neurones the exciting effects of action currents acting across synapses are exerted during the synaptic delay, i.e. if such action currents are the synaptic transmitters, their delayed action in setting up the discharge of an impulse indicates the presence of an intermediate response of the nerve cell. Thus, on either hypothesis of synaptic transmission, detonator summation would involve summation of subliminal responses set up by preganglionic impulses at different synapses. This summation must be mediated by a rapid propagation through the cell of these subliminal responses, and, when these responses thus sum to a certain critical intensity, an impulse is set up in the cell and discharged along the axon. The response of the cell which thus mediates the detonator action of preganglionic impulses may be called the "detonator response". As this response is defined solely in terms of the discharge of an impulse by a cell, it is justifiable to assume that this discharge occurs immediately the detonator response attains a certain critical intensity. As we have seen, in some experiments the detonator response set up by a single im-

pulse may be above this critical intensity, but in most experiments it is subliminal.

Since the synaptic delay of the discharge set up by a preganglionic volley is shortened either by a pre-existent C.E.S. [Eccles, 1935*b*; and Figs. 2 and 3] or by a pre-existent detonator response (cf. Figs. 2 and 3), and conversely since it is lengthened by the refractory period following an antidromic volley [Eccles, 1936*b*], the detonator response must have a

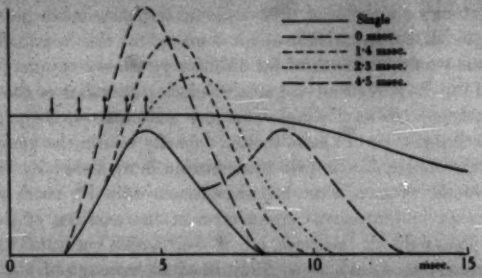

Fig. 4. Diagram showing for a single ganglion cell the presumed course of summation of the detonator responses at the various stimulus intervals of Fig. 2. The zero of the time scale (abscissa) is the calculated time of arrival of the fastest preganglionic impulses at the synapses. The threshold intensity at which the detonator response sets up an impulse is shown by the horizontal line, the decline of this line beyond 6 msec. showing the diminution of the threshold by the developing C.E.S. This diminution would reach a maximum at about 15–20 msec., and return to normal would occur at about 200 msec. When the rising detonator response crosses the threshold line, an impulse is set up (indicated by the arrow pointing upwards), and the consequent refractory period prevents any further determination of the course of the detonator response. Its subsequent course is thus purely hypothetical. The arrows pointing downwards indicate the calculated time of arrival of the fastest impulses of the second preganglionic volley at intervals of 1·4 and 2·3 msec. (for zero interval it is at zero time on the graph), the synaptic delay being the interval between such an arrow and the corresponding arrow pointing upwards.

phase of progressive increase to a maximum which is attained not later than the end of the longest synaptic delay, i.e. with S_1 ganglion cells at about 4–5 msec. after the arrival of the earliest preganglionic impulses at the synapses. From the following argument it will be seen that the variations which occur in the detonator summation of two preganglionic volleys at various intervals, e.g. in Fig. 2, suggest that the detonator response in a single ganglion cell has a time course which is approximately shown by the continuous line in Fig. 4. The line initially running horizontally

represents the threshold intensity at which the detonator response sets up an impulse, hence the single detonator response is subliminal in that particular ganglion cell, which is thus in the "subliminal fringe". Other ganglion cells would be excited by a single preganglionic volley to varying degrees of subliminality or supraliminality, and the conditions for these various cells may be inferred from the special case depicted in Fig. 4. In constructing the curves (Fig. 4) for the summed detonator responses at the various stimulus intervals of Fig. 2, the detonator responses for each volley are assumed to be identical and to sum arithmetically. These curves illustrate the experimental finding that the second volley sets up a response with the shortest synaptic delay when there is an interval of about 2 msec. between the two volleys (cf. Figs. 2 and 3). With shorter stimulus intervals, the increased height of the summed detonator response explains the observed larger spatial facilitation, and the simultaneously observed decrease in temporal facilitation is also illustrated in Fig. 4. Again, with simultaneous volleys, Fig. 4 shows that there would merely be an increase in steepness of the already steep rising phase of the detonator response; hence an explanation is provided for the small shortening then observed in the synaptic delay, an exactly similar effect being produced by increasing the size of a single preganglionic volley. On the other hand, the rapid decline shown for the detonator response in Fig. 4 must be assumed in order to explain the observed absence of detonator facilitation (cf. Fig. 2) that is illustrated in Fig. 4 for an interval of 4·5 msec. Finally, the considerable synaptic delay (about 2 msec.) which occurs even under optimal conditions suggests that, as shown in Fig. 4, the detonator response only attains a significant intensity at about this interval after the calculated time of arrival of the preganglionic impulses at the synapses.

The curve shown in Fig. 4 for the detonator response also forms the basis of the following explanations which may be offered for other experiments on synaptic delay and facilitation in sympathetic ganglia.

(1) The late beginning for the detonator response is also suggested by the temporal facilitation produced by pre-existent C.E.S. [Eccles, 1935*b*]. Just as with detonator summation, there is a limiting value of about 2 msec. below which the synaptic delay cannot be shortened. Fig. 5 illustrates the experimentally observed effect of C.E.S. in producing both temporal and spatial facilitation by lowering the threshold at which the detonator response sets up the discharge of an impulse (cf. section B), the temporal facilitation being larger with those ganglion cells which normally have the longest synaptic delays. Thus in Fig. 5 the ganglion cell normally

excited just to threshold intensity has its synaptic delay shortened by 1·5 msec., while the cell excited to twice normal threshold has its delay shortened by only 0·6 msec.

(2) Fig. 6 illustrates the observed action of antidromic impulses in lengthening the synaptic delay [cf. Eccles, 1936*b*, p. 13, section A]. The continuous curve shows the course of the detonator response which normally would set up an impulse at R_1, which corresponds to G_3 in Fig. 1. After an antidromic volley the raised threshold of the relatively

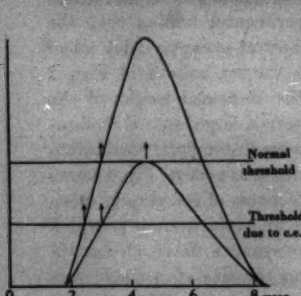

Fig. 5.

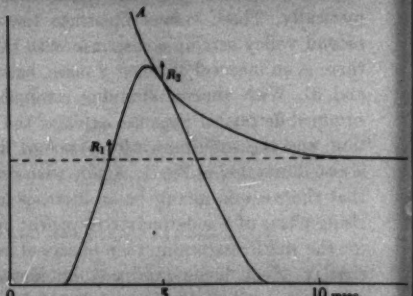

Fig. 6.

Fig. 5. As in Fig. 4, but to show the effect of the lowered threshold (due to C.E.S.) on the synaptic delay of two ganglion cells, one whose detonator response just attains threshold intensity, and the other with double this intensity.

Fig. 6. As in Fig. 4, but to show how the raised threshold following an antidromic impulse delays the time at which an impulse is set up by the detonator response.

refractory period [cf. Brown, 1934; Eccles, 1936*b*] recovers approximately along the line A, the antidromic volley in the figure being critically timed so that this line just touches the detonator response at R_2. Any further retardation of the antidromic volley prevents the detonator response from ever attaining threshold, hence R_2 represents the latest time at which the detonator response can still set up an impulse from that ganglion cell, i.e. R_2 corresponds to G_4 of Fig. 1. Fig. 6 in addition shows how the antidromic experiments of section A give evidence of the rapid decline of the detonator response.

(3) It has been shown that the synaptic delay of S_2 ganglion cells is not diminished, when, during its latter part, there is a further excitation of these cells by preganglionic impulses incident at synapses different from those primarily excited [Eccles, 1935*a*, p. 201]. Hence, after the arrival

of this secondary group of impulses, there must be a latent period of several milliseconds before any appreciable detonator response is produced. The conditions are illustrated in Fig. 7 in terms of the postulated detonator response curve which has, however, been modified in order to accord with the longer synaptic delays observed for S_2 ganglion cells.

Thus the time course suggested for the detonator response in Fig. 5 provides a satisfactory explanation of all experiments on facilitation and synaptic delay, and on no other basis does a satisfactory explanation seem possible.

The experiments of Lorente de Nó [1935*a*, *b*, 1936] on the oculomotor neurones provide an exact parallel to these observations on the ganglion cells of the superior cervical ganglion [cf. Eccles, 1936*c*,

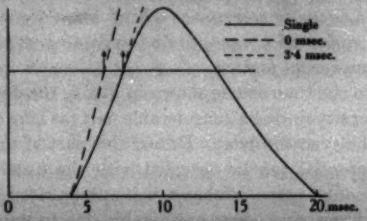

Fig. 7. As in Fig. 4, but to illustrate, with S_2 ganglion cells, the shortening of synaptic delay which is produced by a second preganglionic volley at the indicated intervals after the first. With simultaneous volleys the synaptic delay (measured from zero of the time scale) is shortened by about 1·2 msec., while, with a stimulus interval of 3·4 msec., the detonator response of the second volley is too late to affect the synaptic delay.

pp. 374–82], except that the detonator response runs a time course of about one-fifth the duration, the synaptic delay for these neurones being correspondingly about one-fifth of that for the S_1 ganglion cells. Again, with motoneurones of the spinal cord, it has recently been shown [Eccles & Pritchard, 1937] that there is an initial quick detonator response setting up the discharge of impulses with a synaptic delay of about 0·7–1·0 msec., the first development of C.E.S. again not being detectable until just after this discharge. The time course of the detonator response and the synaptic delay are here, too, almost five times shorter than with S_1 ganglion cells. *Thus it would seem likely that excitatory impulses incident at the synapses of all neurones set up a detonator response having approximately the course shown in Fig. 4, variations in the temporal scaling corresponding to the synaptic delays characteristic of these various types of neurone.*

Moreover, both in oculomotor neurones and in motoneurones of the spinal cord there is summation of the detonator responses produced at different synapses on a neurone. A detonator response must, therefore, be propagated rapidly from the region of the synapse where it is produced. Such a propagation of a subliminal excitatory condition can have no relation to the propagation of all-or-nothing impulses; nor would it seem, itself, to be all-or-nothing in character, for, in those ganglion cells where no detonator summation is demonstrable, the detonator response presumably disappears before reaching the regions of other synapses. Again, during an experiment lasting for several hours, detonator summation often is observed to diminish gradually (cf. Figs. 3 and 9*a*], an effect presumably resulting from the experimental interference. *Thus we have the picture of the detonator response as a brief excitatory state produced in a neurone at the region of a synapse and spreading rapidly through that neurone in a decremental fashion.*

According to the time course shown in Fig. 5, the detonator response increases in intensity during a considerable part (as long as 2 msec.) of the latter part of the synaptic delay. During this part of the synaptic delay no electrical potential can be detected with the usual leads from the ganglion cells, the first potential change being propagated directly along the postganglionic fibres, and hence presumably due to impulses discharged from the ganglion cells. Thus it would seem that the detonator response does not give rise to any detectable electrical change between the soma and the axon of a ganglion cell. In this respect the detonator response resembles the local excitatory state of peripheral nerve [Blair & Erlanger, 1936]; and in other respects also they appear to be closely related excitatory conditions [cf. Eccles, 1936*c*, p. 360].

The presumed time course of the detonator response in Fig. 5 shows that only the early part of the synaptic delay precedes the beginning of the detonator response, the latter part being occupied by the rising phase of this response. This initial part, which may be as long as 2 msec., undoubtedly is partly due to slowed conduction velocity in the very fine terminal branches of the preganglionic fibres, no allowance being made for this in calculation of the time of arrival of the preganglionic impulses at the synapses. The remainder would be due to the time which preganglionic impulses take to set up a detonator response after they have reached the synapses, and so may be occupied partly in the rising phases of the preganglionic impulses themselves, partly in transmission of their excitatory action to the ganglion cell, and partly by the delay experienced by such an action in setting up the detonator response. Thus the synaptic

delay is compounded of the times occupied in the following events [cf. Eccles, 1936*c*, p. 383].

(1) Delayed conduction of impulses in the fine terminal branches of the preganglionic fibres.

(2) Duration of the rising phases of the preganglionic impulses themselves.

(3) Synaptic transmission from the preganglionic fibre to the ganglion cell.

(4) Delay incurred by the synaptic transmitter in setting up the detonator response.

(5) Time occupied by the rising phase of the detonator response in attaining threshold intensity for setting up an impulse. Presumably this rising phase is conditioned partly by the asynchronism in the time of arrival of preganglionic impulses, and partly by the conduction time (and hence summation time) of the detonator responses generated at the different synapses of a ganglion cell.

E. The synaptic transmitter

In section A the term "detonator action" was applied to that brief excitatory action by which preganglionic impulses set up a discharge of impulses from ganglion cells. Thus it includes the "detonator response" of the ganglion cell, but in addition it must also include the means by which impulses in the preganglionic fibres exert their excitatory action across the intercellular region of the synapse, i.e. the "synaptic transmitter". According to one hypothesis this transmitter is the acetylcholine which is secreted by the synaptic regions of the preganglionic fibres and which diffuses to, and excites, the ganglion cells. According to the alternative hypothesis the action currents of the preganglionic impulses directly excite the ganglion cells at their synaptic regions.

Now, on either hypothesis, the detonator response of the ganglion cell is produced by the action of the synaptic transmitter, which, consequently, must have a duration at least as brief as that demonstrated for the detonator response, i.e. about 6 msec. Such a brief duration presents no difficulty to the action-current hypothesis, for, according to this hypothesis, the synaptic transmitter, i.e. the action currents, cannot have a duration of more than a few milliseconds. With the acetylcholine hypothesis, however, such an extremely rapid inactivation of the secreted acetylcholine could conceivably occur only by the hydrolysing action of cholinesterase. Now the cholinesterase of the ganglion is inactivated by eserine, the acetylcholine secreted from the preganglionic terminals then

accumulating in the ganglion and appearing in the perfusate [Feldberg & Gaddum, 1934; Feldberg & Vartiainen, 1934; Brown & Feldberg, 1936]. If the synaptic transmitter is acetylcholine, it would therefore be expected to have a much more prolonged action after eserinization of the ganglion, but the following experiments show that this does not happen.

(1) Action potentials from the ganglion and from the postganglionic trunk show no alteration in the time courses either of the spike potentials or of the late potential waves (the *N* and *P* waves). An increase in the spike height of submaximal responses is produced by small concentrations of eserine, an effect which is due to the increased excitability and the consequent discharge of impulses by many ganglion cells previously in the subliminal fringe [Feldberg & Vartiainen, 1934; Eccles, 1935*b*, p. 222]; but the important points are, firstly, that the time course of the spike is not altered, i.e. there is no increase in the temporal dispersion of the discharge, and secondly, that there is no detectable repetitive discharge of impulses [Eccles, 1934; Bronk *et al.* 1935].

(2) This absence of a repetitive discharge has been confirmed by recording from the nictitating membrane, and repeating the experiments of section A after eserinization of the ganglion. Fig. 8 is typical of such experiments (five in all) and shows that a suitably timed antidromic volley, e.g. in Fig. 8 one set up 8 msec. after the preganglionic volley, still completely blocks all the impulses which are discharged from the ganglion cells in response to a single preganglionic volley. This latter volley, therefore, does not set up more than one impulse from any ganglion cell.

(3) The plotted points of Fig. 8 provide a more delicate test for any effect that eserine might have on the synaptic transmitter. As the antidromic volley is set up at progressively shorter intervals after the preganglionic volley, i.e. as A_1 is moved progressively further to the left in Fig. 1, the preganglionic volley finds that more and more ganglion cells have so far recovered from their refractoriness that a discharge of impulses can be set up from them. Fig. 8 shows that eserinization of the ganglion (0·4 mg. per kg. intravenously) makes no significant difference in the time course of this recovery curve. Now eserine also does not appreciably alter the refractory period of the ganglion cells (as observed by direct electrical recording from them), hence, by applying the argument of section A, it may be concluded from Fig. 8 that eserine also has no appreciable effect on the time course of the detonator response set up by the synaptic transmitter of the preganglionic volley. Therefore, presumably, the duration of the synaptic transmitter itself is also not significantly lengthened by eserine.

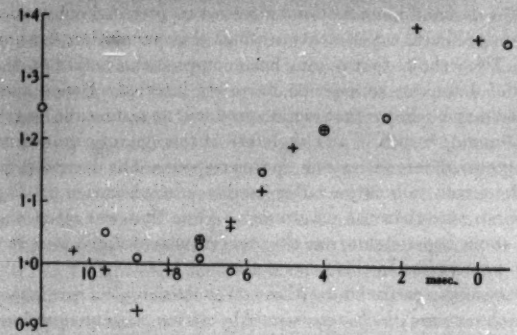

Fig. 8. A maximal antidromic volley is set up at various intervals *after* a maximal preganglionic volley, and the contraction tensions of the nictitating membrane are plotted as ordinates (measured as a fraction of the average tension produced by the antidromic volley alone) against the corresponding stimulus intervals as abeciss*æ*. At intervals of 7–10 msec. the preganglionic volley produces no significant change in the contraction. The crosses show observations before and the circles after the intravenous injection of 0·4 mg. eserine per kg.

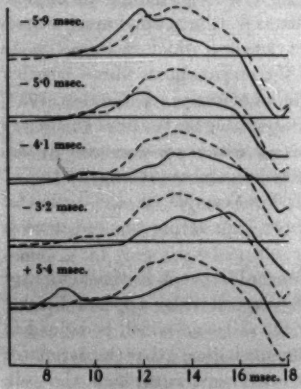

Fig. 9 *a*.

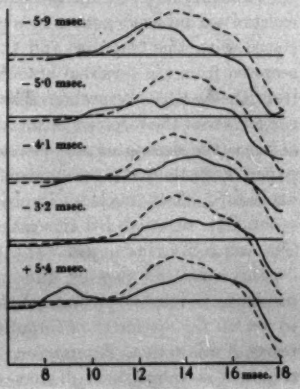

Fig. 9 *b*.

Fig. 9 *a*. As in Fig. 3, and later in the same experiment.

Fig. 9 *b*. A series of observations just after those of Fig. 9 *a*, 0·4 mg. eserine per kg. being given between the two series of observations.

(4) The detonator summation between two preganglionic volleys has been examined in six experiments in which this summation was normally present. Fig. 9 shows that eserine has no appreciable action on the relationship of detonator summation to volley interval. Hence again the conclusion may be drawn that eserine produces no significant lengthening of the detonator response, and therefore of the synaptic transmitter by which a preganglionic volley sets up this response. The decline in the size of the detonator summation after eserine administration in Fig. 9 is probably no more than the progressive decline that was often observed in many of our experiments, e.g. the observations of Fig. 3 were recorded about an hour earlier in this experiment.

The foregoing experiments would seem to establish beyond reasonable doubt that the cholinesterase which is inactivated by eserine plays no significant part in bringing about the rapid decay of the synaptic transmitter, and therefore provide a very grave difficulty for the hypothesis which regards this transmitter as acetylcholine. The alternative action-current hypothesis thus receives indirect support. Two recent reviews [Eccles, 1936*c*, pp. 366–70; Eccles, 1937] may be referred to for a discussion of these results in relation to the acetylcholine hypothesis.

F. Conclusions

There are two possible mechanisms by which a preganglionic impulse exerts its excitatory action on a ganglion cell—the action currents associated with this impulse, and the acetylcholine which it causes to be secreted from the terminal branches of the preganglionic fibre—possibly from the *boutons* themselves. The available evidence [cf. Eccles, 1937] suggests that the former mechanism is responsible for the brief excitatory actions (the detonator response and C.E.S.) which are discussed in this paper, while the latter mechanism possibly has a more prolonged "tonic" excitatory effect, such as is observed, for example, in the action of adrenaline secreted by the motor fibres innervating smooth muscle [Eccles & Magladery, 1937*b*].

However this may be, the next detectable event is the detonator response, an excitatory state of the ganglion cell which appears directly to set up the discharge of impulses by the cell, and which is defined in terms of this action. Summation experiments indicate that the detonator response is propagated decrementally and rapidly through the cell, summation of the responses produced at different synapses thus being produced. These experiments also show that detonator responses of various degrees of subliminality may be produced in ganglion cells (the subliminal fringe). When thus too weak to set up the discharge of an impulse, the

detonator response disappears within a very few milliseconds. If, on the other hand, the detonator response sets up an impulse, its subsequent course is submerged by the consequent refractory period. The brief duration of the detonator response similarly precludes the demonstration of detonator summation by successive volleys in the same preganglionic fibres.

The more prolonged excitatory state, C.E.S., must be sharply distinguished from the detonator response. In ganglion cells its sole action appears to be a lowering of the threshold at which the detonator response sets up the discharge of an impulse. It does not begin until about the end of the detonator response, its relation to this response being illustrated in Fig. 4. However, such a delayed and prolonged course must not be regarded as evidence for the existence of a more prolonged type of synaptic transmitter, for the C.E.S. set up by an antidromic impulse, i.e. in the absence of a transmitter, runs an identical time course [Eccles, 1936*b*, section D]. Since C.E.S. always follows the detonator response, and since summation between different synapses has never been observed for C.E.S. when not preceded by an initial facilitation wave due to detonator summation (cf. Figs. 2 and 3), it seems likely that C.E.S. is not set up directly by a synaptic transmitter, but is produced secondarily to the detonator response. The setting up of the detonator response would then be the only primary action of the synaptic transmitter, but such a conclusion must be regarded as merely provisional, pending further experimental evidence.

Summary

By analysing experiments on the interaction of preganglionic and antidromic volleys, it is shown that a preganglionic volley sets up the discharge of an impulse from a ganglion cell (an explosive act) by exerting on it a very brief excitatory action, called the "detonator action". The "detonator action" must include the synaptic transmitter which mediates the transmission across the intercellular region of the synapse, but it also includes the response which this transmitter sets up in the ganglion cell, the "detonator response", which may be of varying intensity, the discharge of an impulse being, by definition, instantaneously produced when a certain critical intensity is attained.

Experiments on summation of the excitatory actions of two separate preganglionic volleys at different intervals show that this detonator response is a brief excitatory event which is rapidly propagated (apparently decrementally) from the site of its production to sum with the detonator responses produced on neighbouring synapses of a ganglion cell. Analysis of such summation indicates that the detonator response reaches a sig-

nificant intensity about 2 msec. after the calculated time of arrival of the preganglionic impulses at the synapses, attains a maximum about 2 msec. later, and then rapidly decays. This time course is shown to be in agreement with other experiments on facilitation and synaptic delay, and synaptic delay is discussed in relation to the detonator response.

The brief duration of the detonator response, and hence of the synaptic transmitter causally related to this response, is not significantly lengthened when eserine inactivates the cholinesterase of the ganglion. A grave difficulty is thus presented to the hypothesis which regards acetylcholine as the synaptic transmitter. On the other hand, the alternative "action-current" hypothesis accords well with the experimental observations of this paper, for according to it the synaptic transmitter is the action current of each preganglionic impulse and so necessarily would be of short duration and unaffected by cholinesterase inactivation.

The C.E.S. set up by a preganglionic volley runs a much slower time course than the detonator response. Facilitation experiments show that it does not begin until after the end of the synaptic delay, and it increases to a maximum at about 10–15 msec. later. C.E.S., therefore, can have no *direct* effect in setting up the discharge of impulses, its sole action being a lowering of the threshold at which the detonator action of later preganglionic impulses sets up such a discharge, i.e. it produces an increase in the explosiveness of the ganglion cells.

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THE ENERGY LIBERATION AT CONSTANT DIASTOLIC FIBRE LENGTH IN THE TORTOISE HEART WITH PARTICULAR REFERENCE TO THE EFFECT OF THE EMPTYING PRESSURE

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Considerable doubt was cast upon the validity of the generalization of Starling & Visscher [1927] that the energy liberation of the isolated heart "maintained under constant chemical and temperature conditions, is determined by...the initial length of its muscular fibres", by the assertion of Stella [1931] that at constant initial fibre length the energy output varies with the arterial resistance. The latter author believed that, in addition to the initial length factor, a work factor entered into the determination of total energy liberation in contraction.

It should be noted that Stella's results were obtained with the tortoise ventricle, whereas those of Starling & Visscher were with the dog's heart in the heart-lung preparation. Several investigators have confirmed the Starling & Visscher results in the mammalian heart [Hemingway & Fee, 1927; Gremels, 1933; Peters & Visscher, 1936] and in the amphibian [Clark & White, 1928, 1930; Eismayer & Quincke, 1930; Decherd & Visscher, 1933, 1934]. According to Stella, all other investigators worked over a limited range of pressures and, therefore, failed to observe the relation he described. As a matter of fact, Starling & Visscher [1927] studied pressures from 60 to 180 cm. H_2O and were not able to go to lower pressures because the coronary blood flow falls off greatly below the lowest figure mentioned. Stella's [1931] pressure variations were between 15 and 60 cm. H_2O, which is not a relatively larger range. Stella found an increase in energy liberation at constant diastolic fibre length of 25 p.c. when the pressure was increased from 15 to 45 cm. H_2O. With the method he used this extent of change should be significant, and since he states that twenty-three out of twenty-

nine experiments gave such results, it would appear that some importance should be attached to the observations if they could be confirmed. This paper is a report of an attempt to confirm the observations of Stella. It will be shown that the conclusions of Stella are based upon a methodical error, and that when the latter is obviated there is a constant relation between initial fibre length and energy liberation when chemical and temperature conditions are held constant, regardless of changes in mechanical factors.

Decherd & Visscher [1933] have already pointed out that the interpretation of Stella that his results represent a sort of "Fenn effect" [Fenn, 1923] is untenable, because Fenn found a higher energy liberation in isotonic contraction in which work was performed than in isometric contraction. Decherd & Visscher [1933] showed that with an approach toward isometric contraction by an increase in arterial pressure the oxygen consumption, representing the total energy liberation, remained invariably a function of diastolic fibre length. There was never an exception to this rule in their experiments. Decherd & Visscher did not, however, repeat Stella's experiments in every detail, and consequently could not refute his contention that over wide variations of pressure the energy liberation varies with the work performed at constant diastolic volume.

The first apparent difference between the tortoise and the dog heart is that at very low rates of contraction it is possible to vary the arterial pressure against which the tortoise ventricle is made to pump without altering the diastolic volume, whereas this is practically impossible with the mammalian heart. Stella [1931, p. 254] says, "In each experiment the diastolic volume was kept constant by adjusting the venous pressure to such a level that a period of diastasis should always precede the onset of systole; under these conditions the diastolic intraventricular pressure at the end of diastole remained the same, as long as the height of the venous column was not altered." We [Moldavsky & Visscher, 1933] showed that it was only an exceptional tortoise heart which would behave in this manner at rates of contraction between 5 and 30 per min., temperatures between 10 and 30° C., and venous pressures between 5 and 25 cm. H_2O. Only one heart out of twenty so studied showed filling independent of the residual fluid in the ventricle (which was varied by changes in arterial pressure) even at such low heart rates as 10 per min. at 20° C. It should be noted that precautions were taken to have as little resistance as possible in the venous circuit. Consequently, we were unable to confirm Stella's conclusion that, in the average tortoise heart in good condition, the diastolic volume is independent of the emptying (or arterial)

pressure at the heart rates, venous pressures and temperatures which he used. This point is of some significance in harmonizing our present findings with his, since because of his assumption that diastolic volume is dependent only upon venous filling, and is independent of arterial pressure, as quoted above, he may have depended too heavily upon this relationship. Moreover, as will be seen, his method of measuring diastolic volume was itself faulty.

Methods

The oxygen consumption of the isolated ventricle of the tortoise (American sand turtle) stimulated to contraction at the frequencies indicated in each experiment by break-induction shocks was measured by the apparatus described by Decherd & Visscher [1933]. The apparatus consists essentially in the device described by Stella [1931] except that the oxygen uptake from the exterior of the heart is neglected and that the valves in the perfusion system were of a different type. In attempting to use the valves as described by him, we were unable to obtain free flow with low-pressure gradients, but this can be obtained readily with the valve we used. Carbon dioxide was absorbed from the venous reservoir serating chamber by means of isotonic sodium hydroxide in porous porcelain suspended in the chambers. The volume of the ventricle was determined by connecting the heart chamber with a calibrated tambour,[1] and recording the diastolic and systolic volumes under the conditions of filling and artificial resistance for any particular observation. Simultaneous continuous measurements of ventricular volumes and oxygen consumption are possible by this method and are not by that of Stella.

The perfusion fluid consisted of a mixture of one part of turtle's blood with ten to twenty parts of Ringer's fluid. The oxygen tension in the venous reservoir was maintained at approximately 700 mm. of mercury, and the oxygen content of the fluid was measured in four experiments before and after work periods, in order to make sure that an oxygen deficit in the perfusion system might not produce errors in interpretation. The oxygen content by the Van Slyke-Neill method [Van Slyke & Neill, 1924] was between 1 and 2·0 volumes p.c. in different experiments (see Table I) and did not alter significantly over periods of one hour, during which contraction continued.

The intraventricular pressure was measured by means of a sensitive membrane manometer, calibrated for each experiment.

[1] The tambour was calibrated in position in the system as a whole by adding fluid from a microburette to the heart chamber. In this way the pressure-volume relationship in the calibration is identical with that in the actual measurement.

TABLE I. Oxygen in perfusion fluid, volumes p.c.

Exp. no.	Before work period	After work period
1	1·12	1·27.
2	1·47	1·48
3	1·23	1·23
4	1·10	1·05

TABLE II

Exp. no.	Intraventricular pressure (cm. H_2O) Systolic	Diastolic	Ventricular volume (c.c.) Systolic	Diastolic	Output c.c./beat	Work g.cm./hr.	Oxygen consumption c.c./hr.	Total energy liberated g. cm./hr.	Efficiency W/E p.c.
1†	20	0·0	0·08	1·35	1·27	16,000	1·80	389,000	4·1
1†	35	10·0	0·30	1·35	1·05	24,000	1·84	397,000	6·1
1†	45	13·0	0·75	1·35	0·60	18,000	1·68	406,000	4·4
2*	55	8·0	0·00	3·60	3·60	152,000	5·60	1,210,000	12·5
2*	60	10·0	1·35	3·60	2·25	101,000	5·60	1,210,000	8·3
2*	65	10·0	1·95	3·60	1·65	82,000	5·60	1,210,000	6·7
3	45	5·0	0·15	3·00	2·85	93,000	2·68	579,000	16·6
	55	6·0	0·60	3·00	2·40	106,000	2·68	579,000	18·4
	60	7·0	1·50	3·00	1·50	70,000	2·68	579,000	12·8
	65	8·0	2·40	3·00	0·60	22,000	2·64	570,000	3·8
4	35	6·0	0·60	3·45	2·85	74,000	2·60	565,000	13·1
	45	8·0	0·90	3·45	2·55	85,000	2·60	565,000	15·1
	50	8·0	1·65	3·45	1·80	66,000	2·60	565,000	12·1
			The same heart at a lower venous pressure						
4	35	0·0	0·45	2·85	2·40	76,000	1·80	389,000	19·2
	40	2·0	0·50	2·85	2·35	80,000	1·80	389,000	20·5
	20	5·0	1·67	3·75	2·08	28,000	1·80	389,000	7·1
	35	8·0	1·95	3·75	1·80	44,000	1·83	395,000	11·2
	55	20·0	2·55	3·75	1·20	38,000	1·80	389,000	9·7
6	20	5·0	0·60	3·90	3·30	45,000	1·53	331,000	13·5
	45	10·0	1·50	3·90	2·40	75,000	1·53	331,000	22·6
	55	11·0	2·25	3·90	1·65	65,000	1·56	337,000	19·2
7	20	1·0	0·00	3·53	3·53	60,000	1·59	343,000	17·4
	38	3·0	0·90	3·53	2·63	83,000	1·62	350,000	23·7
	45	7·0	1·80	3·53	1·73	59,000	1·56	337,000	17·5
8	13	3·0	0·60	1·80	1·20	11,000	1·05	227,000	4·8
	30	5·0	0·90	1·80	0·90	20,000	1·05	227,000	8·8
9	15	3·0	0·30	1·20	0·90	10,000	1·29	279,000	3·5
	25	5·0	0·45	1·20	0·75	14,000	1·35	292,000	4·6
	30	6·0	0·60	1·20	0·60	13,000	1·32	285,000	4·4
9	30	11·0	2·40	4·40	2·00	34,200	3·90	843,960	4·1
	45	14·0	3·20	4·38	1·18	32,922	4·20	908,980	3·6
	40	12·0	2·60	4·40	1·80	45,360	4·50	973,800	4·7
9	10	2·5	2·20	3·00	0·80	5,400	1·80	389,520	1·3
	38	4·0	2·30	3·15	0·85	25,770	2·10	454,440	5·7
	30	3·0	2·25	3·00	0·75	18,465	2·10	454,440	4·1
10	40	3·0	3·60	6·80	2·80	93,240	2·70	584,000	16·0
	48	5·0	4·20	6·80	2·60	88,920	2·70	584,000	15·2
11	20	12·0	2·80	3·80	1·00	7,200	2·55	552,000	1·3
	40	15·0	3·00	3·80	0·80	18,000	2·52	545,000	3·3
	28	13·0	2·92	3·80	0·88	11,880	2·58	558,000	2·1
	20	12·0	2·80	3·80	1·00	7,200	2·55	552,000	1·3
12	30	10·0	2·00	6·00	4·00	72,000	2·10	454,000	15·8
	45	12·5	2·40	6·00	3·60	105,300	2·13	461,000	22·9
	35	11·5	2·40	6·00	3·60	72,900	2·16	467,000	15·6

Contraction rate was 15 per min. in all experiments. Temperature was maintained at 20° C. in all experiments excepting those marked †, which were at 16° C., and those marked *, which were at 27° C.

Results

The pertinent experimental data from twelve complete experiments in which oxygen consumption, ventricular volume, and intraventricular pressure were measured at several levels of arterial pressure are presented in Table II. In order to maintain the diastolic ventricular volume constant with increasing arterial pressure produced by increasing the peripheral resistance, it was always necessary to reduce venous filling pressure.

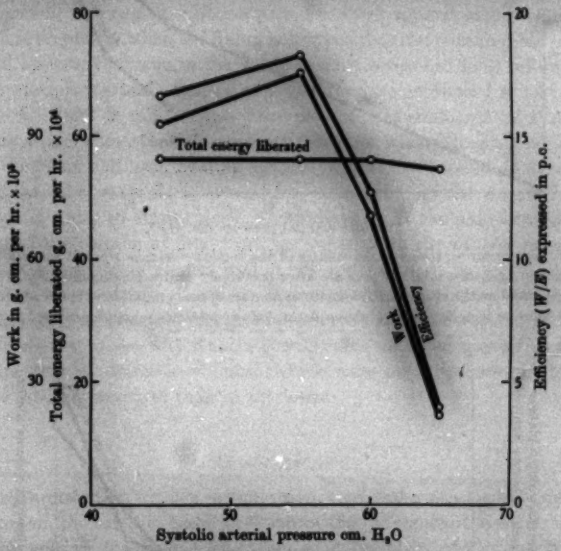

Fig. 1. Total energy, work and efficiency in the turtle's ventricle in relation to ejection pressure. Rate 15 per min. Temp. 20° C.

There are small differences in oxygen consumption indicated in some experiments with alterations in systolic pressure. In no case is the change greater than 5 p.c., and the variation is entirely random in different experiments. In no instance is there any change of the magnitude observed by Stella, and we attribute the small fluctuations that were observed entirely to random error in measurement. In different experiments we have covered a larger pressure range than that used by Stella, 10–65 cm. H_2O. It is interesting to note that our results at high arterial pressure

confirm the previous study [Fenn, 1923] in which it was found that the work diminished as isometricity of contraction was approached, but the energy liberated remained constant, as shown in Fig. 1.

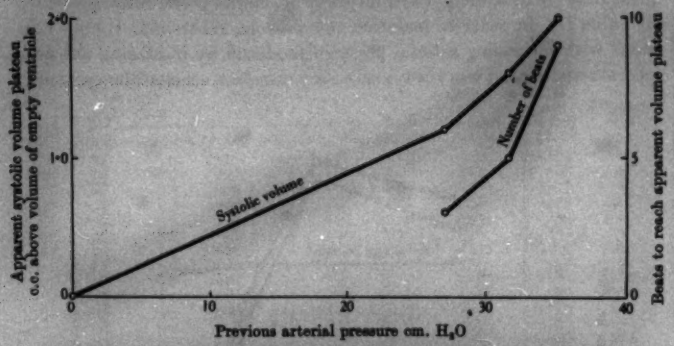

Fig. 2. Upper curve: true systolic volume of the turtle's ventricle when it was allowed to empty against a minimal pressure after previously contracting against the pressures indicated on the abscissa. Lower curve: number of beats required to reach an apparent minimum systolic volume (volume plateau) after contracting against indicated pressures.

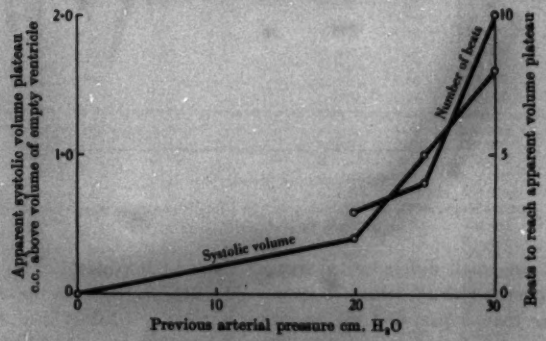

Fig. 3. Results of experiment comparable to that in Fig. 2. Curves labelled similarly.

We find no evidence, therefore, that the energy liberation of the tortoise ventricle is determined by any other factor than the diastolic volume, which is a measure of the initial fibre length.

Stella's method of measurement of ventricular volume appears to have led him into error. After closing the heart chamber by clamping off the connecting tube to the venous reservoir, he recorded the ventricular volume by noting the change which occurred after clamping the venous return tube and releasing the arterial pressure clamp. It seemed plausible that the ventricle would completely empty itself in a few beats and attain the empty systolic volume, regardless of what the previous arterial pressure had been. The heart muscle, however, is subject to a type of hysteresis in this regard. After a period of work against high pressures, complete emptying when the filling is stopped requires considerable time, and an apparent plateau in the volume curve is reached long before the empty state is reached. Several experiments were performed to test this question. Figs. 2 and 3 show examples of measurements of systolic volume, in c.c. above that of the empty ventricle, when filling was stopped after the heart had been beating against various resistances. It must be noted that the ventricle is always forced to beat against a certain resistance in order to empty itself. This resistance at the minimum is the weight of the column of fluid from the ventricle to the venous reservoir outlet, approximately 15 cm. H_2O in our apparatus.

Since the ventricle shows an apparent volume plateau at higher volumes after working against great pressures than after working against low, it is easy to see why Stella should have obtained greater oxygen consumption at diastolic volumes which were actually greater at the higher arterial pressures than at the lower.

Conclusions

Measurements of oxygen consumption, ventricular volume and arterial pressure on the tortoise heart have shown that at constant diastolic ventricular volume the energy liberation is independent of the maximum systolic pressure. The latter was altered by changes in peripheral resistance and the diastolic volume kept constant by adjustments in venous filling pressure.

These results fail to confirm the statement of Stella that, in the tortoise ventricle, the energy liberation increases with arterial pressure at constant diastolic volume.

It has been observed, further, that after contracting against higher pressures the ventricle comes to an apparent systolic volume plateau at a level higher than the volume of the empty ventricle. Considerable time is required for the ventricle to empty itself against minimal pressures under

such circumstances. This property of heart muscle was not taken into account by Stella, and this, it is believed, accounts for the apparent discrepancy between his findings and those of others.

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A SIMPLE DIRECT-COUPLED AMPLIFIER FOR ACTION POTENTIALS

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A previous paper [Garceau & Forbes, 1934] described a direct-coupled amplifier for use with a string galvanometer, adapted to slow responses such as action potentials of smooth muscles and glands. This has served in a number of researches and has proved useful. The complexity of its construction and operation and the resulting trouble and expense of maintenance have led us to look for a simpler method of attaining the same result. The amplifier here described is presented not as embodying the highest refinement attainable in direct-current amplification, but as a practical apparatus which is cheap and easy to construct and has proved adequate for a variety of physiological purposes.

In all direct-coupled amplifiers the problem of balancing battery-voltage decays is usually met by a bridge circuit of one type or another. In the push-pull amplifier the tubes and the plate resistors are the source of potential (Fig. 1). In other cases the batteries are the bridge arms, as in the amplifier herein described (Fig. 2). The advantages of the latter method lie in the simplicity of the balancing circuits and the elimination of the necessity of finding tubes which have very similar characteristics.

In addition to choosing a bridge circuit which is simple and not critical to balance, it is possible to choose tubes which are relatively independent of battery voltages. The pentode voltage amplifying tubes are most desirable, since their plate voltage may be varied over wide ranges without changing their plate current appreciably. This means that $I_p R_p$, the voltage which is applied to the grid of the next stage, is nearly constant as E_b drifts. If change of screen voltage causes significant drift, the screen can be biased from an independent battery, from which no appreciable current is derived. In practice, we find that the major source of drift is in the heater-cathode circuit, which can be made small by using

a large lead storage battery to heat the filament, in addition to choosing a filament that operates above the saturation point of the I_a–E_f curve. Commercial tubes that have these general characteristics are RCA-38 and 6C6. British tubes with similar characteristics are Osram VMP4G and MSP-4, respectively; these, however, have not actually been tried.

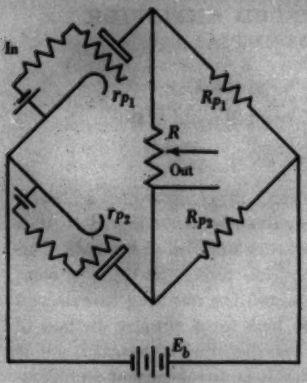

Fig. 1.

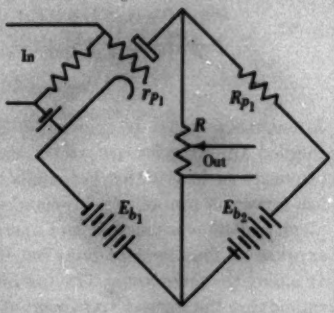

Fig. 2.

Fig. 1. The push-pull stage represented as a bridge circuit shows that the condition for balance is $\frac{r_{p_1}}{r_{p_2}}=\frac{R_{p_1}}{R_{p_2}}$, where r_p is the dynamic plate resistance. In direct-coupled stages the additional static plate requirements are that $\frac{R_{dc_1}}{R_{dc_2}}=\frac{R_{p_1}}{R_{p_2}}$, where $R_{dc}=\frac{E_p}{I_p}$ and $R_{dc_1}=R_{dc_2}$. Therefore R_{dc} must equal r_p in each tube. This condition is not so easy to fulfil.

Fig. 2. The conditions for D.C. balance in this case are $\frac{E_{b_1}}{E_{b_2}}=\frac{r_{p_1}}{R_{p_2}}$. The dynamic balance is fulfilled, since the internal resistance of the batteries is about zero in both cases; hence any balance that is good for the D.C. is balanced for all conditions.

Another problem always encountered in direct-coupled amplifiers is the difficulty of coupling the first plate circuit into the grid of the following tube. Where high-voltage plate supplies are employed, this difficulty of balancing the following grid circuit becomes very prominent. If we choose the plate battery voltage of the first stage, E_b, small, E_{R_p} also becomes small, and the difficulty in balancing the following grid circuit is lessened. Another advantage of using low plate voltages in low-level amplifiers is the elimination of noise caused by the ionization of residual gas within the tube [Johnson & Neitzert, 1934]. Operation at those low voltages

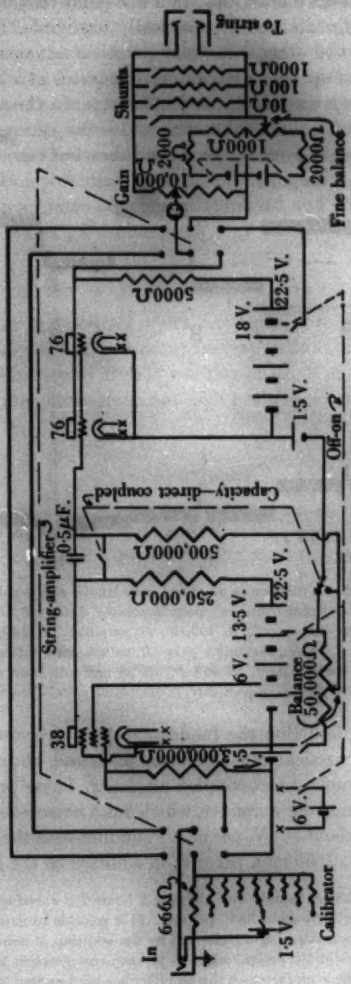

Fig. 3. Circuit diagram of the amplifier. Ω = 1 ohm.

reduces the plate battery drain to such a low point that battery-voltage decay, besides being balanced, is practically negligible. The use of low plate voltages has two other important practical advantages—elimination of the hazard of operating laboratory apparatus at a high potential, and economy in both installation and upkeep, since the small dry-type battery is sufficient. The only point sacrificed is the gain per stage, which is reduced by a factor of 3 or 4 in the above tubes, but can still be made as high as 30, as in this amplifier.

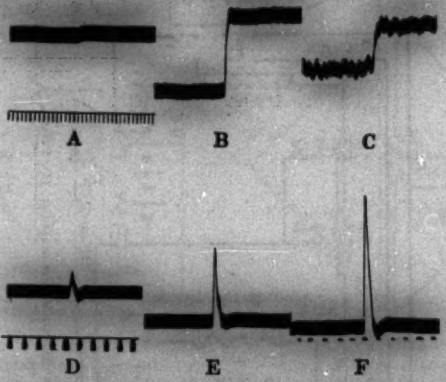

Fig. 4. Records showing performance of amplifier with Hindle string galvanometer. Resistance of string 3500 ohms; optical magnifications: *A–C*, 500; *D–F*, 400. *A–C*, 10,000 ohms in series with input. *A*, 100μV., no amplifier. *B*, 100μV., amplifier at 19 p.c. of full gain. *C*, 10μV., maximum gain. *D*, submaximal action current of cat's peroneal nerve, no amplification. *E* and *F*, action currents from same nerve with same stimulus at 7 and 14 p.c., respectively, of maximum gain. Time below in 10 msec.

It will be recognized that the fundamentals of this circuit itself are not new, but the practical considerations discussed above permit the design of a very simple direct-coupled amplifier. These principles were applied to the design of our amplifier, which has a noise level of 1 or 2μV. peak and a drift of about 10μV. per min.,[1] and increases the sensitivity of recording from 15 to 1000 mm. per mV. in addition to the gain obtained

[1] By using a large storage battery and allowing 2 hours for warming up, it has been possible to reduce this drift to about 5μV. per min. It is possible to reduce the drift still further by the use of more balancing circuits and by the addition of more tubes, but this would defeat the purpose of the design, which is to keep complication and expense at a minimum. For most of the work for which this amplifier has been wanted, these refinements have not been deemed worth while.

by increasing input resistance from that of the string (e.g. 3500 ohms) to that of the grid leak, which may be made as high as 3 megohms without materially increasing the noise level. This last feature has been very helpful in recording from microelectrodes whose resistance is necessarily large. By throwing a single switch, the amplifier may be made capacity-coupled to eliminate fortuitous slow potential changes.

In Fig. 4 are shown calibration records of constant currents and examples of action potentials in physiological material, records taken from nerve affording a comparison of the amplified and unamplified response with the same string galvanometer.

SUMMARY

An amplifier is described which has the following advantages: It is simple and cheap to build; it occupies very little space; it is very simple to operate and is easy to keep in working order; it eliminates the need of working with the galvanometer at high positive potential; and it affords a steady base line.

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THE SENSATION OF YELLOW AND ANOMALOUS TRICHROMATISM

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In 1881 Rayleigh described a condition which he called anomalous trichromatism, meaning that the individuals so affected had apparently normal trichromatic vision but that they made anomalous mixtures of wave-lengths 6700 and 5350 A. in order to match a light of fixed wave-length of 5890 A. Out of twenty-three subjects, sixteen agreed in the ratios of "red" to "green" required to match the "yellow" standard colour. Of the remaining seven, two required relatively more "red", and five relatively more "green" to match the same "yellow". He stated that the colour vision of all his subjects was normal, but he did not mention in what way, if at all, they had been tested. Later observers confirmed and extended these results [Parsons, 1924].

Göthlin [1923] recorded the fact that the region of the spectrum which gives rise to the sensation of yellow varies in extent and in position in different individuals. We thought it worth while to compare the extent and position of the "yellow" region with the ratios of "red" to "green" required to match a fixed "yellow". If the fixed "yellow" appeared orange to one and green to another, the former may require relatively more "red" and the latter relatively more "green" to match the fixed wave-length.

Methods

In order to measure the extent and position of the portion of the spectrum which gives rise to a yellow sensation, one collimator of the spectroscope previously described was used [Roaf, 1927]. The shadow of a needle, placed vertically above the slit, was reflected by a mirror, above and in the same plane as the grating, and the image was focussed on a millimetre scale 200 cm. from the axis of rotation. The readings of the scale were converted into wave-lengths by the use of a table. This table

was constructed by means of the formula for a grating placed at right angles to the telescope of the spectroscope ($n\lambda = b \sin \phi$: Baly, 1924), and by trigonometrical conversion of angles into millimetres on the scale. These values were checked by measurement of certain lines in the neon vapour and copper arc lamps.

The slit was illuminated by a hundred watt gas-filled tungsten filament lamp.

The subject was asked to set the slit in the telescope of the spectroscope so that the light appeared yellow. He was then told to move the telescope until the light appeared to be just tinged with orange. Finally the subject was asked to move the telescope in the opposite direction until the faintest tinge of green was visible. Five readings on each side of the yellow were recorded: ten readings in all. In this way the range of the spectrum which gives rise to the sensation of yellow was determined. The mid-point between the faintest tint of orange and that of green should be the purest yellow, or, as it will be called in this paper, "mid-yellow".

The observations are similar to the measurement of hue discrimination [Corbett, 1936] only there is no fixed wave-length with which a comparison can be made: the readings show that part of the spectrum which would be called yellow by the various subjects.

For the readings of the colour mixture a "Nagel anomaloscope" was used. This consists of a direct-vision spectroscope in which the field of view is divided into a lower and upper field. The lower field is a spectrum formed from a single slit, of which the intensity can be varied by altering the width of the slit by means of a graduated screw. The upper field is formed from two superimposed spectra from two slits. A graduated screw moves a tongue between the slits, so that at one extremity of its movement the first spectrum is obliterated and only the second is visible, whilst at the opposite extremity the second spectrum is obliterated and only the first is seen. At intermediate positions the two spectra are mixed in varying proportions.

The top slit corresponding to the lower field is placed so that the wave-lengths visible are from the region about 5890 A. The two bottom slits corresponding to the upper field show wave-lengths of 6700 and 5350 A. respectively. The positions of these slits were standardized by the flame spectra of sodium, lithium and thallium respectively. The aperture of the eyepiece permits one to see the fields by the Maxwellian view.

The slits of the spectroscope were illuminated by a hundred watt gas-filled tungsten filament lamp. The subject adjusted the lower drum until the two fields appeared of the same colour, and then the upper one until the

two fields were of the same brightness. Adjustments were continued until the subject was satisfied that the two fields were identical in hue and in brightness. The readings on the drums were recorded, the settings altered and the subject repeated the matchings until five separate observations had been made. The records therefore are the results of five separate readings of the proportions of "red" and "green" to match a fixed "yellow", and five corresponding readings of the relative brightness of the mixture as judged by the intensity of the pure "yellow" light.

The colour vision of all the subjects was tested by the Ishihara pseudo-isochromatic plates [Ishihara, 1932], and any subject making an abnormal reading either of the position and extent of the yellow region or with the anomaloscope was further tested by a lantern described by Corbett & Roaf [1937].

Results

Owing to the large number of figures to be recorded, space does not permit of their publication in tabular form, but the full results are recorded in a thesis by Hailwood [1935].

The first ten subjects were more or less trained individuals, and each was examined on ten separate occasions in order to see whether the readings differed from day to day in the same subject.

It was found that both the width of the "yellow" band and the position of the "mid-yellow" varied from day to day. The width of the "yellow" band was less variable than the position of the "mid-yellow" wave-length.

The results for the ten trained subjects are shown in Table I, the variability of the results being shown as the standard deviation which is given with all the averages in this paper. The variations in extent of the yellow region might have been due to differences in muscular sense of

Table I. Summary of results on ten individuals each observed on ten separate occasions

Subject no.	Width of the yellow band		Position of mid-yellow		Anomaloscope mixture		Intensity of yellow	
	Median	Average	Median	Average	Median	Average	Median	Average
1	59·5	63·2 ± 13·10	5824·0	5826·6 ± 17·8	61·5	61·4 ± 1·5	32·1	31·9 ± 1·2
2	63·0	61·8 ± 6·75	5897·0	5901·0 ± 10·9	60·7	60·4 ± 1·3	32·5	32·6 ± 0·6
3	175·5	200·6 ± 88·00	5614·5	5613·4 ± 84·5	80·0	80·1 ± 1·2	20·0	19·2 ± 0·6
4	40·0	39·2 ± 4·50	5822·0	5824·2 ± 9·6	66·0	66·0 ± 0·7	30·0	30·3 ± 1·0
5	34·0	35·4 ± 11·73	5818·0	5822·9 ± 15·0	63·0	63·1 ± 0·7	32·7	32·9 ± 1·2
6	185·5	207·3 ± 68·30	5928·0	5916·8 ± 75·5	31·7	31·2 ± 3·0	37·1	37·1 ± 1·3
7	122·5	118·9 ± 38·20	5836·0	5840·0 ± 24·9	66·1	66·0 ± 1·2	30·2	30·2 ± 0·8
8	90·5	98·4 ± 17·90	5839·0	5839·0 ± 12·2	68·0	67·9 ± 1·0	29·4	29·8 ± 0·9
9	31·0	29·9 ± 5·70	5796·0	5786·0 ± 5·3	65·0	65·0 ± 1·4	34·4	34·3 ± 1·4
10	72·5	73·8 ± 19·80	5812·0	5814·0 ± 16·9	65·4	65·2 ± 1·5	34·4	34·3 ± 1·0

Nos. 3 and 6 had defective colour vision.

these trained subjects, but the subjects had been told to observe the colour, not the extent of the movement of the screw to produce a change in colour. Furthermore, after each reading the experimenter altered the setting, so that the subject did not have the same starting-point on which to base his muscular movement.

The readings of the anomaloscope also differed from day to day. In every case the daily value given is the median reading of the five separate readings. This figure is preferable to the average, as it is less affected by a single aberrant result such as may be obtained from time to time in measurements of sensation.

Although observations on trained subjects are more reliable the observations were extended to ninety other subjects, all medical students, each of whom made the series of five readings for each value on one occasion only. The median reading is used throughout.

Two of the ten trained subjects and eleven of the ninety additional subjects were found to have defective colour vision. The remaining eighty-seven subjects made no mistakes with the Ishihara plates and any one of them who made readings of the yellow region greater than 125 A. or anomaloscope readings outside the usual range was tested further by a lantern test. In view of the absence of any proof that these subjects had any defect in colour vision, they were considered to have normal colour vision.

Fig. 1 shows the position and extent of the yellow region of eighty-seven normal subjects plotted against the readings of their anomaloscope mixtures. By adding the number of lines above the wave-lengths at intervals of 20 A., the total number who would agree in calling those respective wave-lengths yellow is obtained. Fig. 2, in which these numbers are plotted, shows a general resemblance to a "probability" curve. Of the eighty-seven normal subjects, fifty-two or 59·8 p.c. agree in recognizing 5840 A. as yellow, whilst the arithmetical mean of the series is 5853 ± 38.

Fig. 3 shows the position and extent of the "yellow" region for the thirteen subjects with defective colour vision plotted against their anomaloscope readings. In order to place all the results on one diagram the scale of the anomaloscope readings is much less than in Fig. 1. The average value of the position and extent of the yellow region and of the anomaloscope readings for the eighty-seven normal subjects is shown as a line surrounded by a rectangle, the sides of which enclose the standard deviations for the extent of the "yellow" region and for the anomaloscope mixtures. We expected that some of those with defective colour

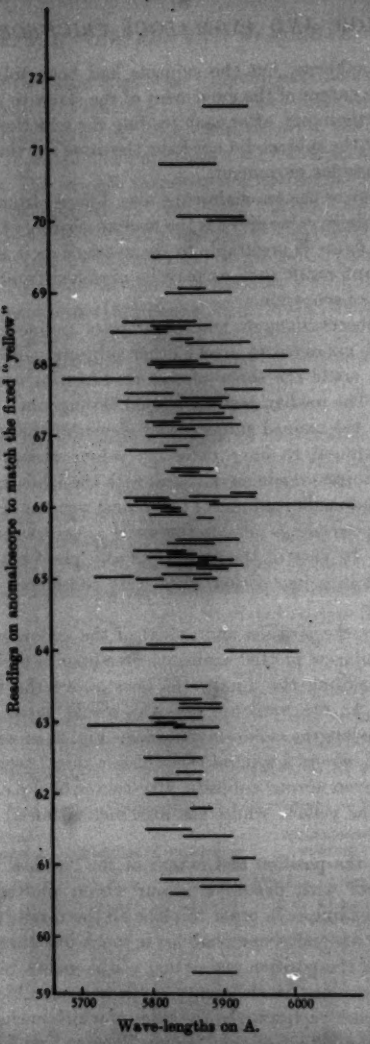

Fig. 1. Graph showing the relation of the anomaloscope readings to the yellow region of eighty-seven normal individuals. Ordinates = readings of the "red"-"green" mixture with the anomaloscope to match the fixed "yellow". Abscissæ = wave-lengths included in the yellow band of the different individuals.

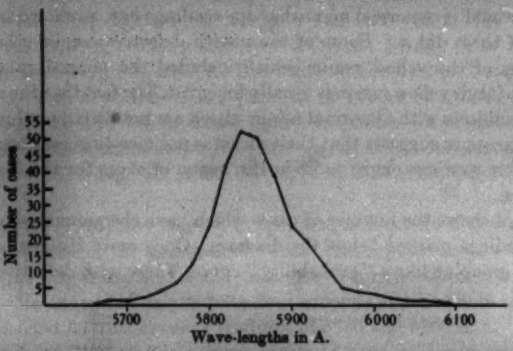

Fig. 2. Graph showing number of normal individuals who agreed in calling certain wave-lengths yellow. Ordinates = number of individuals who included the wave-lengths, shown as abscissæ, in their yellow region.

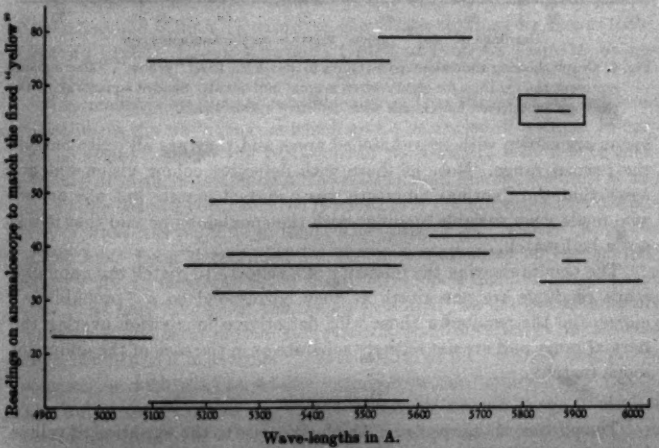

Fig. 3. Graph showing relation of anomaloscope readings to the yellow region for thirteen individuals with defective colour vision. Ordinates = readings of "red"-"green" mixture with the anomaloscope to match the fixed "yellow". Abscissæ wave-lengths included in the yellow band of the different individuals. The average yellow region and the average anomaloscope reading for the eighty-seven normal individuals is shown by a line surrounded by a rectangle which includes the standard deviations of the average length of the yellow region and the anomaloscope readings.

vision would give normal anomaloscope readings but, as shown in Fig. 3, none of them did so. Some of those with defective colour vision gave readings of the yellow region which included the normal range. The extent of their yellow region is usually too great. The fact that the readings by the subjects with abnormal colour vision are not distributed in a haphazard manner suggests that their vision is not merely guesswork, otherwise some readings ought to lie in the region of those for the "normal" subjects.

Fig. 4 shows the number of cases which have the anomaloscope mixture readings marked below the diagram. Once more the normal cases show a grouping like a "probability" curve. Those with defective colour

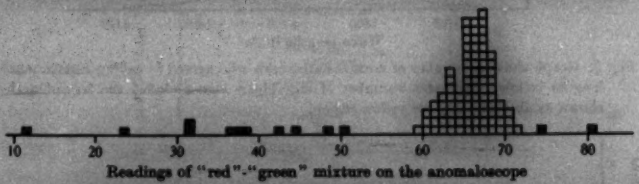

Fig. 4. Graph showing anomaloscope mixtures to match the fixed "yellow". Clear squares represent the readings for eighty-seven normal individuals. Shaded squares show the readings for thirteen individuals with defective colour vision.

vision are shown with cross-hatched areas and they are all quite outside the normal range. Most of those with defective colour vision did not agree that the "normal" mixture was a correct match, but one or two who made very variable readings with the anomaloscope said that it was not a bad match.

The results showing the intensity of "yellow" to match the anomaloscope readings are not given, as they correspond to a "probability" curve, and the results for those with defective colour vision overlap the normal range and are not entirely separate as in the case of the anomaloscope matches.

Discussion

The portion of the spectrum which gives rise to the sensation of yellow is a special region because it marks one of the regions where there is a main maximum of discrimination [Corbett, 1936]. It gives rise to a distinct sensation, thereby differing from the other main maximum which gives rise to a mixed sensation (blue-green).

One result of this investigation is to show the wide range of wavelengths which are seen as yellow by normal individuals. The average

'mid-yellow" for eighty-seven subjects was 5853 ± 38 A. The median figure was 5848 A. with quartiles of 5829 and 5876 A. The extreme readings were 5726 and 5957 A. The width of the "yellow" region was 67 ± 29 A. with a median of 65, quartiles of 46 and 87 A. The extreme values were 17 and 148 A. These figures are not to be compared with those for colour discrimination, as there was no fixed comparison colour. They give the range of what corresponds to the subjects' idea of yellow.

Göthlin [1923] investigated the position and extent of the spectrum which gives rise to the sensation of yellow. He exposed narrow bands of the visible spectrum, and noted those wave-lengths which were called yellow. He examined only twenty-four subjects and he found the mode to be at 5800 A. with extreme readings of 5740 and 5960 A.

Donders [1884] made measurements of pure yellow in seventy-six individuals, and obtained a mean value of 5820 A. with extreme readings of 5720 and 5940 A.

In view of the three results 5853 (Hailwood & Roaf), 5800 (Göthlin) and 5820 A. (Donders), the values obtained by Hess [1889], Listing [1867], Clark-Maxwell [1860] and others seem to be improbable.

The readings for normal subjects suggest that there is a fair degree of variation in the wave-lengths which give a sensation of yellow. It cannot be stated whether the variations in the readings were due to inexperience or misunderstanding of the instructions, but the readings at any one sitting seemed consistent, so it seems as if the subjects had a definite idea of what was required of them. The range of the "yellow" sensation might have been determined by the muscular sense of the subject, causing him to move the slit of the spectroscope a definite distance each time. The only control of this factor is that if one compares the daily readings of the eight normal subjects who gave ten sittings on different days, it looks as if in seven of them the width of the "yellow" region was comparable with the day-to-day variation in the "mid-yellow" values. The only exception was no. 9, who showed a narrow range 31 A. but placed his "mid-yellow" readings at different positions on different days varying from 5684 to 5854 A., whilst his readings on any one day were consistent amongst themselves. It may be that there are individuals who can recognize small differences but do not reproduce the same sensory effect from day to day. It is as if an individual could recognize the differences in two notes sounded simultaneously or successively, but could not place the pitch with any accuracy.

With the anomaloscope used it was found that the average "red"-"green" ratio was 65·65 ± 2·61 and the intensity of the "yellow" required to match this mixture was 33·54 ± 3·01. Whilst persons with defective colour vision might make readings within the normal limits, none of those in our series did so. Any marked departure from the normal limits should suggest defective colour vision.

Houston [1922, 1932] examined 423 men and 104 women, with the result that he found that most of the readings were included in a normal "probability" curve. He therefore agrees with Schuster's [1890] observations. The only point on which we differ from him is that all our cases with anomalous matches had definite defects in colour vision when examined by Ishihara plates and a colour lantern. Houston stated that his aberrant cases "were put through a very stringent test", but he did not state by what method.

Contrary to expectation there was no relation between the positions of the "mid-yellow" of the spectrum and the "red"-"green" mixtures which matched a fixed "yellow". The correlation worked out as recommended by Feldman [1935] amounted to only 0·078 ± 0·06. On the other hand there was a distinct relationship between the "red"-"green" ratio and the intensity of the "yellow" which matched the mixture in brightness. This should be expected because "green" has a greater brightness effect than "red"; the more "red", therefore, there is in the mixture, the less intense the "yellow" that would be required to match the mixture. The correlation coefficient is −0·56 ± 0·05. The negative sign shows that the more "red" in the mixture the less the brightness of it.

Defective colour vision

We began this work in order to discover cases of anomalous trichromatism and in order to compare their anomaloscope readings with the position and extent of their yellow region in the spectrum. Each individual who gave unusual readings with the anomaloscope was found to have defective colour vision, and all those who gave normal readings read the Ishihara plates correctly.

Some of the subjects who gave normal readings with the anomaloscope had a wider range of yellow than usual, and these were examined by a lantern test without proving any defect in colour vision. We recognize that the line between normal and defective colour vision is arbitrarily drawn and that some of the subjects with a wide yellow band may have been borderline cases.

Results obtained since Rayleigh's original observations have tended to show that the frequency of anomalous trichromatism is less than he found in his series of observations.

Our search of the literature leads us to believe that in most records of anomalous trichromatism no proof has been given that the subjects had trichromatic vision. In our series all the anomalous cases definitely had defective colour vision. On the other hand we have not seen any proof that all those with defective colour vision can match all colours by two regions of the spectrum. In a preliminary note Pitt [1935] reports the "coefficient curves" for three anomalous trichromats, but he says nothing about their colour vision. Houston [1922, 1932] points out that the term "anomalous trichromatism" has been used to describe the vision of persons at the extremes of the normal variation and also those well outside those limits. Our experience is that all our cases with matches outside the normal limits had defective colour vision, and we agree with Houston that cases with matching within the range of normal variation should not be considered as separate groups.

In these circumstances we think that it is better to avoid the use of the term anomalous trichromatism unless the subjects have been shown to have trichromatic vision. When an individual has been shown to have defective colour vision, he should be so described and not said to have dichromatic vision unless it has been proved that only two regions of the spectrum are needed for him to match all colours. If persons with defective colour vision do require three regions of the spectrum to match all colours, the third region does not seem to have the same importance and value as for normal people.

It is known that amongst persons with defective colour vision, some see a spectrum of normal extent whilst others have a diminished sensitivity to the "red" end of the spectrum. Guttmann [1907, 1908] has classified hypochromats as red anomalous and green anomalous, but that is confusing as it suggests that they all require three regions of the spectrum to match their complete colour range.

Our interpretation of our anomaloscope readings is that an individual with a diminished sensitivity to the "red" of the spectrum would tend to make an anomalous colour mixture with the "yellow". Persons with normal colour vision adjust the ratio of "red" to "green". Those with defective colour vision are not incommoded by a wrong colour mixture, and they compensate for the lack of balance by a variation in the intensity of the "yellow" required to match the mixture. The latter would appear reddish or greenish to a normal person.

The average "mid-yellow" for the hypochromats was 5547 ± 237 A. with a median of 5488 A. The quartiles were 5365 and 5771 A., with an extreme range of 5089 and 5928 A. The average range was 314 ± 168 A. with a median of 301 A. and quartiles of 177 and 480 A. The extreme values were 35 and 531 A.

The average anomaloscope readings were "red"-"green" ratio 40·8 ± 15·0 and the "yellow" intensity 44·9 ± 15·4. There was a distinct correlation (0·58) between the "red"-"green" ratio and the intensity of "yellow" for those with defective colour vision. The accuracy of the coefficient is not great because of the small number of the observations. The magnitudes of the standard deviations in these cases should be compared with those for the eighty-seven normal subjects.

Summary

1. The range of the "yellow" region and the situation in the spectrum of its mid-position were measured in eighty-seven normal subjects and thirteen hypochromats.

2. The anomaloscope matches of the same one hundred individuals were also measured.

3. Readings were made on ten separate days with eight normal subjects and two hypochromats.

4. The correlation between the positions of the "mid-yellow" wavelength and the anomaloscope readings was found to be insignificant.

5. There was a definite correlation between the "red"-"green" ratio and the intensity of "yellow" required to match the mixture.

6. The significance of the results is discussed with reference to the condition of anomalous trichromatism.

7. There is much greater variability in the measurements made on hypochromats.

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THE INFLUENCE OF THE REACTION OF BLOOD PLASMA ON OXYGEN CONSUMPTION IN RELATION TO THE LAW OF ISODYNAMIC EQUIVALENCE

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That animal metabolism is organized to yield, under standard conditions, a constant expenditure of energy, is a conception which goes back to Liebig, but was first put to the test by Rubner [1883] in experiments mainly designed to demonstrate that when fat is replaced in metabolism by other food substances, the substituted food is burnt in a quantity yielding the same amount of energy. Though Rubner's experiments came, with progress in accuracy, to have only historical value [Tigerstedt, 1909], the isodynamic law is held to be approximately true for carbohydrate and fat over a wide range, chiefly as a result of the investigations of Atwater. The precision with which it is followed has not been established, and the issue is confused by the appearance of so-called specific dynamic effects. Such an effect is so prominent with protein [Rubner, 1902] that it is scarcely possible to speak of an isodynamic law in connexion with it, and the following is limited to a consideration of substitution between carbohydrate and fat.

The isodynamic equivalence might be imagined as resulting from a control of the total heat production in connexion with the regulation of temperature, but as the same body temperature may be maintained under identical external conditions at very different metabolic levels, in myxœdema and in thyrotoxicosis, for example, there is no inextricable association between body temperature and energy production. The isodynamic law might also be conceived as a consequence of the energy expenditure of every organ being fixed by the demands of functional activity. The function, it might be thought, comes first, the energy required for that function is then supplied from whatever sources are possible for the body; and this is the arrangement suggested by the

physiology of active muscle. But even in such a case the energy is eventually supplied by oxidation, and the isodynamic law (in so far as it prevails) implies a control of the oxidation so as to yield the required energy. That the metabolism of all organs is similar in spite of great differences in their function, appears clear from the parallelism of the complex action of temperature on the individual organs with its action on the oxygen consumption of the whole animal. It has been shown that the excitability of the cold end organs of the skin [O'Connor, 1935*a*, 1936*b*], the resting oxygen consumption of muscle [O'Connor, 1936*b*], the functional activity of the kidney [Conway *et al.* 1937] are influenced by temperature in a manner closely parallel with the influence of temperature on oxygen consumption of the body as whole; and the probability of analogous effects on the function of other organs has been pointed out [O'Connor, 1935*b*]. It seems difficult to imagine that this close parallelism could depend on an identical influence of temperature on the diverse functional activities of the separate organs. Rather does it seem likely that the rate of oxidation controls or limits the functional activity, and that the oxidation is itself governed by a system identical in all the cells of the body. The consumption of oxygen depends on and must be largely governed by the nature and concentration of the oxidative catalysts.

The enzymatic processes resulting in oxidation may be summarily described as consisting of a system of dehydrases activating the hydrogen of the different substrates at various stages of metabolic decomposition, together with a system of oxidizing ferments activating or transferring oxygen to the activated hydrogen [see Franke, 1934]. It is through the oxygen activators that the different substrates are united into a common metabolism. There is reason to believe that the oxygen activators rather than the dehydrases control the rate at which the breakdown occurs [Szent-Györgyi, 1924]. If they do in fact control oxidation, they must also control the parallel changes in function, and it is through them that we might most easily conceive a mechanism resulting in the isodynamic equivalence.

In a preliminary communication [Kane & O'Connor, 1936] attention was directed to the possibility that such a regulation might occur through changes in acidity. When carbohydrate is substituted for fat in isodynamic equivalence, there is a fall of about 7 p.c. in the oxygen consumption, but an increase of 33 p.c. in the carbon-dioxide production. This increase of CO_2 production will tend to cause an increase in the hydrogen-ion concentrations of the tissues and of the blood in equilibrium

with them. Further, a more acid blood will be required to maintain the increase in respiratory movements necessary to remove the additional carbon dioxide. It is true that the tendency exists to consider the change in reaction necessary for this to be trivial [Douglas & Havard, 1932], but that it would occur to some extent would be generally admitted. If now on a complete change-over from fat to carbohydrate this alteration in hydrogen-ion concentration, when equilibrium is established, causes a reduction of 7 p.c. in the intensity of the catalytic oxidative activity, the isodynamic equivalence would automatically result for these two substances.

If this be the explanation of the isodynamic law the following two demonstrable consequences follow:

(1) Any artificially produced increase in acidity of the tissues (and blood) should cause a fall in oxygen consumption, no matter what substances are being burnt.

(2) It should be possible to find an association between the respiratory quotient and blood reaction, indicating that an adequate fall in *p*H occurs with rising respiratory quotient.

The first of these points is here dealt with; the second is treated in a subsequent communication.

Indications of the complexity of the control of metabolism have been shown, as already indicated, in the study of the influence of temperature on oxygen consumption. This has been shown, in the anæsthetized animal, to be different in the three temperature ranges 22–29°; 29–34°; 34–40° [O'Connor, 1936*a*]. As reasons exist for believing that the second and third of these phases—though more especially the third—are in action at normal body temperature in the unanæsthetized animal, it was of importance to examine the influence of *p*H on at least these two. Observations in all three phases have in fact been made.

Methods

The examination of the influence of *p*H was done with rabbits under urethane anæsthesia (2 g. per kg.). Urethane alters the course of the metabolic phases, detected by temperature analysis, from their normal relations, but this has at least one advantageous result. The third of these phases normally extends in the rabbit from about 41 to 38° [O'Connor, 1936*b*]. Under urethane it extends down to about 34° before giving place to the second phase. The greater range makes the investigation easier. As it is the most important it has been fully examined.

The rabbits were artificially ventilated and immersed in a bath of saline at a suitable temperature. The artificial respiration was maintained by a Starling "Ideal" pump driving air into the inlet tube of a tracheal cannula. The outlet tube of the tracheal cannula, the dead space of which was made as small as possible, led through a mixing bottle and a Bohr meter to the outlet port of the pump. A continuous sample of the mixed expired air was drawn off throughout the period of observation, and was subsequently analysed. From this analysis, an analysis of the inspired air and the volume of the expired air, the oxygen consumption was determined in the usual way and expressed as c.c. in 5 min. for a rabbit of 2 kg. The determinations (over 20 min. periods) are correct to a coefficient of variation of 3 p.c. [O'Connor, 1936*a*].

The *p*H of the blood was altered in several ways: (*a*) by changing the depth of respiration, (*b*) altering the CO_2 content of the inspired air to air containing CO_2 (up to 8 p.c.) in a Douglas bag connected to the inlet port of the pump, (*c*) by intravenous injections of HCl up to 50 c.c. $N/10$ in 0·6 p.c. saline, (*d*) by intravenous injections of $NaHCO_3$ up to 250 c.c., 5 p.c. Care was taken to vary the sequence of the blood reactions as far as possible.

The *p*H of the blood was determined from the Henderson-Hasselbalch equation in the form

$$p\mathrm{H} = p\mathrm{K}' + \log \frac{[\mathrm{HCO_3'}]}{[\mathrm{CO_2}]} - 0{\cdot}5\sqrt{\mu}.$$

For this equation there was required the partial pressure of CO_2 in the alveolar air and the total CO_2 concentration of the plasma.

The partial pressure of CO_2 was obtained by collecting automatically a small portion (about 0·3 c.c.) of the last of each expiration. This was done in the following way. A mercury filled sampling tube was attached by fine tubing to a side tube on the outgoing branch of the tracheal cannula near its beginning. A few drops of the mercury contained in the sampling tube were allowed to escape at the end of each expiration by an electro-magnet controlled from a cam on the pump wheel. The contents of the sampling tube of alveolar air were subsequently analysed.

The CO_2 content of the plasma was obtained by collecting a sample (about 3 c.c.) of carotid blood at the end of each period of observation. This sample was collected without contact with air under paraffin oil in a centrifuge tube lined with paraffin wax and containing a particle of heparin. The blood was immediately sealed off with molten paraffin wax, cooled on ice and centrifuged. The CO_2 content of 1 c.c. of the plasma was determined by an application of the diffusion method of

Conway & Byrne [1933]. This method, due to Conway & Isaacson (unpublished), consists essentially in allowing the CO_2 to diffuse over from acidified plasma into standard barium hydroxide and titrating. It gives results corresponding to the Van Slyke constant volume gasometric method.

From application of these analyses to the formula the pH was determined. The value of pK′ was taken as 6·33 at 38°, and increased by 0·005 for each degree below this temperature. The dissolved CO_2 at the prevailing alveolar air CO_2 tension and body temperature was determined from the Bunsen solubility coefficient in water as given by a graph drawn from the data of Bohr [1899], altered by a factor so as to correspond with the value of 0·510 for serum at 38° [Van Slyke *et al.* 1928]. The value of $0{\cdot}5\sqrt{\mu}$ was calculated from the average NaCl content of rabbit's plasma and the bicarbonate concentration as given by analysis. It varied between 0·17 and 0·22.

Comparisons with results obtained with the glass electrode give very good agreement at 38°, provided that 15 min. were allowed to establish equilibrium when carbon dioxide was inhaled.

Results

(1) *Influence of* p*H on oxygen consumption at* 36°. For reasons already given the temperature zone to which most attention was directed was that between 35° and 38°. The experiments on this zone are in three groups. In the first group, half-hour intervals were allowed for equilibrium after each change of conditions, and the actual periods during which the oxygen was determined were 20 min. With such times but few observations could be made on each animal. Eighty were obtained on twenty-one rabbits. It then appeared desirable to do a larger number on each animal, lest in seeking to avoid errors arising from uncompleted equilibrium, confusion be produced through the different levels in oxygen consumption in different animals. A second series was done in which the intervals were reduced to 15 min. and the periods to 8. Forty-four observations were obtained on six rabbits. With one of these curare was used. In a third series, in which we had the assistance of Dr O. Fitzgerald, the pancreas with the duodenum or a loop of jejunum was removed 2 hours previous to the first of the observations which were made at intervals similar to the second series. There were seventy-eight observations on seventeen animals.

The results obtained in the second group of observations are given in Fig. 1, in which the data from individual rabbits are indicated by dis-

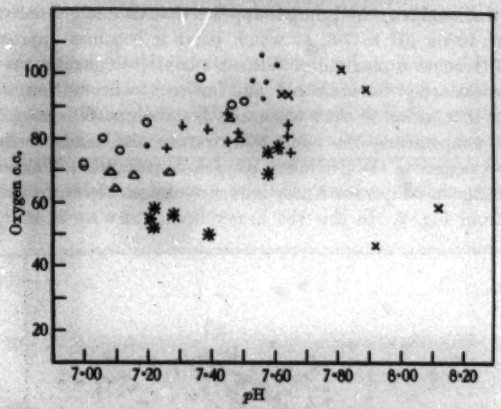

Fig. 1. The influence of acidity on oxygen consumption at about 36°; second group of observations. The separate experiments are indicated by different marks. In the experiment marked by asterisks curare was used.

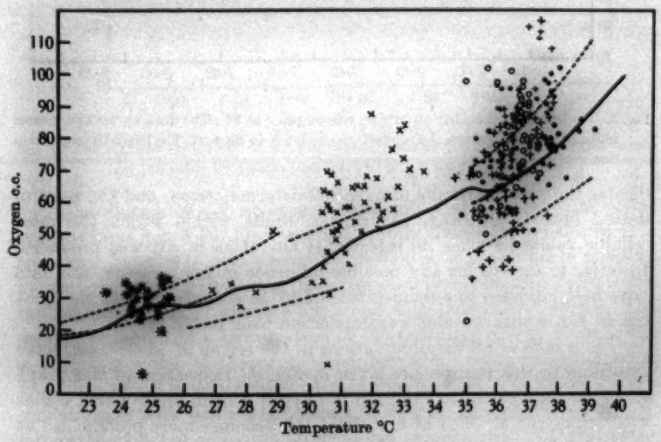

Fig. 2. The relation of the observations on oxygen consumption to rectal temperature (see text). All data were obtained from quiescent rabbits, i.e. shivering was prevented, if necessary, by curare.

tinctive marks. From this group it appears that the oxygen consumption rises with rising *p*H to 7·6, at which point it becomes approximately steady or reaches a maximum. When we come to consider the quantitative relationship between *p*H and oxygen consumption, the first difficulty that arises is that when the metabolism falls in a bath at constant temperature, the body temperature also tends to fall. This tends to exaggerate the decline in oxygen consumption. The extent to which change in temperature may influence oxygen consumption may be judged from Fig. 2. In this the heavy line [Conway *et al.* 1937] in-

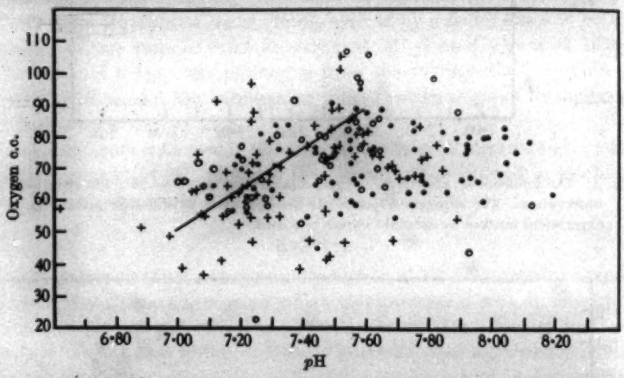

Fig. 3. The influence of acidity on oxygen consumption at 36°. The dots, circles and crosses indicate the three groups of observations alluded to in the text. The line is the regression line.

dicates the average results of over 600 determinations, and the pairs of dotted lines [O'Connor, 1936*a*], the limits within which the most reliable values lie when no intentional alteration in *p*H was produced. In order to correct for any possible influence of temperature, the data have been adjusted to a temperature of 36° in accordance with the thick line in Fig. 2 with the slight extrapolation back to 35°.

The results of all the observations in the region of 35–38°, corrected according to this scheme, are given in Fig. 3. Inspection of this graph gives the impression that the fall in oxygen consumption which begins with falling *p*H at 7·6 is at first slight but becomes more pronounced at about 7·3. This aspect of the result will be considered subsequently, but the immediate interest is to establish the existence of the fall and to measure its average amount as accurately as possible. This was done

by calculating the statistical correlation between oxygen and *p*H up to 7·6.

The statistical results may be summarized as follows: Number of observations, 132. Mean *p*H, 7·32. Standard deviation of *p*H, 0·19. Mean oxygen consumption, 68·5 c.c. Standard deviation of the oxygen consumption, 15. Correlation coefficient, $+0{\cdot}41 \pm 0{\cdot}08$. Regression of oxygen consumption on *p*H, 3·3 c.c. per 0·10 *p*H. From this it appears that a fall of *p*H from 7·4 to 7·3 would produce a fall in oxygen consumption of about 4·5 p.c.

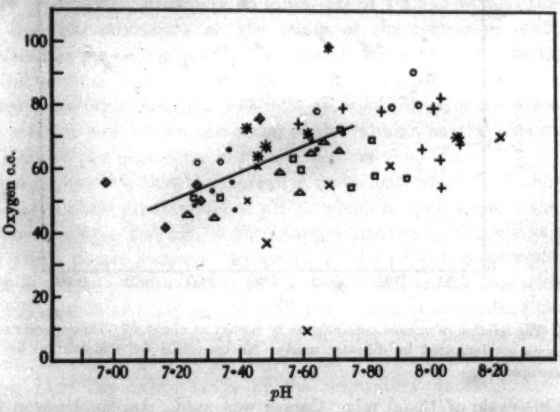

Fig. 4. The influence of acidity on oxygen consumption at 31°. The separate experiments are indicated by different marks. The line is the regression line.

(2) *Influence of* p*H on oxygen consumption at* 31°. Since at rectal temperatures below 34° anæsthetized rabbits almost always shiver, it is necessary, in order to obtain comparable figures, to use curare in addition to the urethane. Fifty-seven observations were obtained from eight rabbits for periods of about 8 min., and with intervals of 15 min. The results, corrected to a temperature of 31° according to Fig. 2, are given in Fig. 4 in which the separate experiments are again distinguishable. The effect of *p*H is very similar to that shown at 36°. The rise in oxygen consumption with rising *p*H appears, it is true, to continue to *p*H 7·7 rather than 7·6, but obviously no stress can be laid on this. The statistical analysis of the influence of *p*H up to 7·74 gives the following: Number of observations (excluding the one very low value from a moribund rabbit),

39. Mean and standard deviation of *p*H, 7·46 ± 0·18. Mean and standard deviation of oxygen consumption, 61 ± 11·5 c.c. Correlation coefficient, +0·61 ± 0·10. Regression of oxygen, 3·9 c.c. per 0·1 *p*H. A change from *p*H 7·3 to 7·2 will cause a fall of about 6·5 p.c.

(3) *Influence of* p*H on oxygen consumption at* 25°. Different from the two previous cases are the results obtained at 25°. There are twenty-six observations on five rabbits obtained during periods of 9–17 min. and

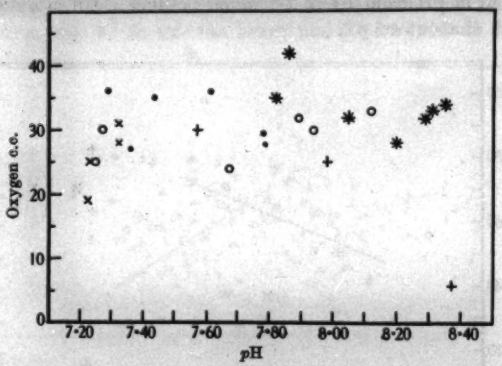

Fig. 5. The relation of oxygen consumption to acidity at about 25°. The separate experiments are indicated by different marks. No correction for temperature has been attempted.

with intervals of 15–50 min. Curare was used. As can be seen from Fig. 5, in which the separate experiments are distinguished, *p*H between 7·2 and 8·3 has no effect on oxygen consumption. Attempts to drive the *p*H outside these limits caused death of the animal.

Discussion

If the activity of organs be associated with their oxygen consumption and if, further, the rate of oxidation be in the main governed by the active concentration of the oxidative catalysts, it follows that the oxidative catalysts control the fundamental activity of organs, i.e. the energy expended in them. From this it follows—omitting the unlikely possibility that the energy consumption controls the concentration of the catalysts—that the isodynamic equivalence must result from an influence on the oxidative catalytic system. If the suggestion put forward in the communication, that this control is exercised through the in-

fluence of the blood (and tissue) *p*H be correct, the first condition is that the blood *p*H must be below 7·6, as, above this, *p*H has no marked effect on oxygen consumption. In fact in the rabbit 7·6 is just above the upper limit of usual arterial *p*H which, according to Hawkins [1924], is 7·58. In man the highest normal value seems to be 7·52 [Earle & Cullen, 1929]. If now we assume that when fat and protein were being metabolized, 15 p.c. of the oxygen was used for protein, and that, this remaining unchanged, the fat was completely replaced by carbohydrate, the oxygen consumption should fall 6 p.c. for complete constancy of energy production. Assuming an initial *p*H of 7·4 and using a range of four standard deviations as the limits of the regression coefficient calculated as prevailing at 36°, it appears that the oxygen consumption will fall between 3 and 5·8 p.c. for a fall of 0·1 *p*H. For isodynamic equivalence a fall of between 0·11–0·20 *p*H would therefore be necessary. Such a fall is well within the range of the rabbit's normal [Hawkins, 1924], and is not impossible in man.

It is improbable that the regression coefficient, which is of course an average over the whole range of *p*H to which it applies, gives the true relation of oxygen and *p*H. A better approximation to this will be given by a graph drawn through the means of the oxygen consumption in Fig. 3 in arrays of 0·10 *p*H. If this is done it will appear that a change as small as 0·08 at a *p*H of 7·30 would be sufficient to produce the isodynamic law. *p*H differences of this amount have been met with in man in the course of a single day during which there is no reason to believe that the entire range of the respiratory quotient has been covered [Cullen & Earle, 1929; Shock & Hastings, 1934]. There is consequently no improbability involved in the theory that the fall in oxygen consumption on a carbohydrate diet may be produced by a fall in *p*H. Evidence pointing to the existence of a fall of this type will be dealt with in a following communication. That the effect of acidity on oxidation may be produced through an influence on catalysts, gains in plausibility from the fact that *p*H has a similar influence on catalysts known to be involved in oxidations, such as indophenol oxidase [Gräff, 1922], and succinicodehydrase [Cook & Alcock, 1931].

There is a corollary argument arising out of the facts presented above which suggests a further test of the theory. At a temperature of 25° *p*H has no influence on oxygen consumption. If now the oxygen consumption of cold-blooded animals be controlled at this temperature by the same catalytic system—and inspection of the observations of Benedict [1932] on the CO_2 production of reptiles indicate that this is so—

it would follow that, at this temperature, cold-blooded animals would not exhibit the isodynamic law. Data are, however, not available for this test.

Summary

The intensity of oxidation in the body must depend in the first instance on the concentration and activity of the oxidative catalysts. As the law of isodynamic equivalence, applied to carbohydrate and fat, implies a diminution of the amount of oxidation when carbohydrate is being oxidized, it is suggested that this decrease is due to an influence on the oxidizing catalysts of a more acid reaction of the blood and tissues resulting from the increased production of carbon dioxide during carbohydrate oxidation.

The influence of artificial alteration of the *p*H of the arterial blood on the oxygen consumption of the anæsthetized rabbit was examined at three temperatures. At 36° and at 31° a fall of *p*H below about 7·6 causes a fall in oxygen consumption of approximately 5 p.c. for a fall in *p*H of 0·1.

For isodynamic replacement of fat by carbohydrate a fall of *p*H of at least 0·08 or more probably 0·11–0·20 would be necessary. Changes in *p*H of this order may occur normally.

At 25° variation in *p*H between 7·2 and 8·3 has no influence on oxygen consumption.

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THE RELATION BETWEEN RESPIRATORY QUOTIENT AND ALVEOLAR CO_2 TENSION

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In a foregoing communication [Kane & O'Connor, 1937] it was suggested that the basis of the constancy of the energy production of the body lies in the constancy of the oxidizing power of the tissues. The energy value of the main constituents of the body is approximately uniform when comparison is made with volume of oxygen used rather than the weight of substance burned. The rate of oxygen consumption must depend very largely on the concentration of the catalysts mediating the oxidation. That it would also be influenced by the concentrations of the substrates is highly probable but the complexity introduced by this "plethora" effect must for the sake of clarity be ignored here. If the concentration of the oxidizing catalysts be constant, the rate of oxidation will approximate to constancy. The approach to isodynamic equivalence in the replacement of carbohydrate for fat is, however, closer than would be achieved if the consumption of oxygen were kept constant. The energy value of oxygen when used in carbohydrate combustion is 7 p.c. greater than that for fat. If the oxygen consumption were strictly constant, the energy yield of the body would be 7 p.c. higher when carbohydrate replaced fat. The closer approach to isodynamic equivalence would, however, be reached if the activity of the catalysts were correspondingly lowered by some chemical influence acting during carbohydrate combustion. The final difference between carbohydrate and fat in combustion is the larger quantity of CO_2 produced from carbohydrate. If this increase in CO_2 results in a more acid blood, and if the acidity causes a decrease in the activity of the oxidizing ferments, complete isodynamic equivalence might be reached or approached.

It has been shown that an acid reaction lowers the amount of oxygen consumption in rabbits at normal body temperature, and that the

necessary change in *p*H for isodynamic equivalence is of plausible amount (at the lowest estimate 0·08, but more probably between 0·11 and 0·20) [Kane & O'Connor, 1937]. It remains to seek evidence that the *p*H of the blood changes in the required direction and extent when carbohydrate replaces fat.

If the bicarbonate concentration of the blood plasma remains constant, any change in *p*H of the arterial blood will show itself as a change in the partial pressure of CO_2 in the alveolar air. If the blood becomes more acid during carbohydrate combustion, there should be a rise in the CO_2 tension, and it should be possible to demonstrate a statistical association between the R.Q. and CO_2 tension in man. The original observations of Haldane & Priestley [1905] indicated that the alveolar CO_2 tension is constant, and this was subsequently taken as indicating that the reaction of the blood was kept constant [Hasselbalch, 1912]. When it was noticed that the alveolar CO_2 tension did not remain constant but changed with food [Hasselbalch, 1912; Dodds, 1921; Endres, 1922], the theoretical constancy of *p*H being considered established, alteration in CO_2 tension was explained by changes in the bicarbonate concentration consequent on the secretion of acid gastric or alkaline pancreatic juice. It appeared subsequently, however, that the plasma bicarbonate concentration did not vary [Dodds & McIntosh, 1923], that the HCl secretion by the gastric juice was not sufficient to produce the change required and was in any case largely balanced by the simultaneous secretion of pancreatic juice [Brunton & Israëls, 1930], and finally that blood *p*H was not constant, but varied by as much as 0·08 or 0·10 during a routine day, during which the total CO_2 remained comparatively constant [Cullen & Earle, 1929; Shock & Hastings, 1934].

There is then no reason to consider it improbable that changes in the alveolar CO_2 tension represent changes in reaction. It is true that under conditions of unusual or abnormal acid production in muscular work or ketosis this does not hold, and that changes in diet are believed to produce confusing effects [Hasselbalch, 1912], but none of these considerations is relevant to the subsequent observations. These were planned to be made on ourselves to determine what relation, if one exist, could be found between the R.Q. and the carbon dioxide tension. As it seemed unlikely that a sufficiently wide range of R.Q.'s could be got under basal conditions, the observations were made in the forenoon and afternoon over a period of 6 months under routine dietetic conditions.

Details bearing on the metabolic conditions are as follows:

Fitzgerald, age 26. Height, 177 cm. Weight, 70 kg. Breakfast; one egg, toast, butter, marmalade. Mid-day meal usually contained meat. A few observations were taken fasting; before others 100 g. of sugar were taken. Number of observations, 55.

O'Connor, age 51. Height, 170 cm. Weight, 78 kg. Breakfast similar. Mid-day meal, 150 g. biscuits and tea. On two occasions a meat meal was taken at mid-day, and on two others additional carbohydrate. Number of observations, 63.

Method

Precaution was taken that no notable exercise was taken for some time before the observation, which consisted in the following routine. A sample of alveolar air from the expiratory position was taken and analysed. Immediately after this the subject sat down to an apparatus consisting of a mouthpiece leading to a mixing bottle of 3 litres' capacity and thence to a gas meter. The mixing bottle was connected to a sampling tube which could be filled with the mixed expired air by allowing the mercury to flow continuously from it through a capillary tube furnished with a tap. The subject breathed through this apparatus for 3 min., during which time the sampling vessel was filled with expired air so as to wash out all connexions. At the end of this period the sampling tube was again filled with mercury from below and breathing through the apparatus resumed. When at least 15 litres of air had passed through the apparatus, the actual observation was started. A stopwatch was started at a noted reading of the gas meter, and the collection of the sample of expired air begun by opening the tap on the mercury outflow. At the end of 5 min. the observation was concluded by closing off the taps on the sampling tube and noting the reading of the gas meter. Complete quiescence was maintained throughout, except from movements involved in starting and finishing the observations. Duplicate analyses were subsequently made of the air sample, and from the mean of these, together with the usual readings of volume, temperature and pressure, the oxygen consumption and CO_2 production were determined. At the conclusion of the respiratory sample a second alveolar air sample was taken from the inspiratory position. The partial pressure of the alveolar air was calculated from the mean of the two.

Results

In Fig. 1 the relation between the R.Q. and the partial pressure of CO_2 is given. This shows clearly, for both subjects, a rise in the CO_2 tension with rising R.Q. As the interest centres round the change in pH which falls in proportion to the increase in the logarithm of the CO_2

tension—the arterial plasma bicarbonate being assumed constant—statistical calculation of the relation between R.Q. and the *logarithm* of the CO_2 tension was made with the following results: Number of observations, 118. Mean and standard deviation of R.Q., $0{\cdot}817 \pm 0{\cdot}062$. Mean and standard deviation of the logarithm of CO_2 tension, $1{\cdot}635 \pm 0{\cdot}023$. Correlation coefficient, $+0{\cdot}398 \pm 0{\cdot}08$. Regression coefficient of log (CO_2 tension), $+0{\cdot}015$ per $0{\cdot}1$ R.Q.

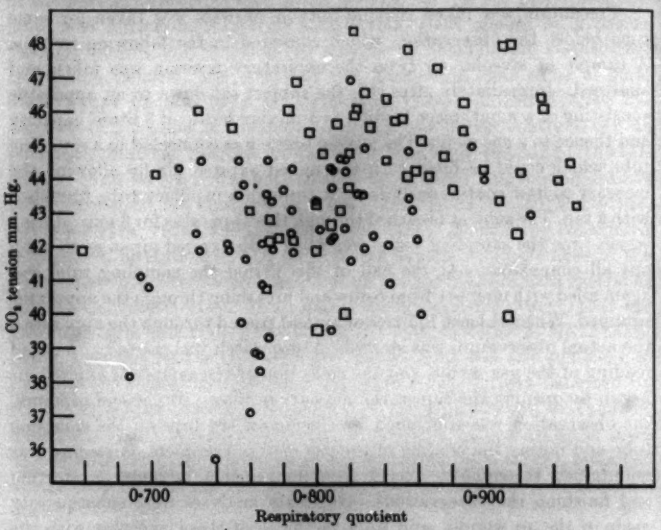

Fig. 1. The relation of the tension of CO_2 in alveolar air to R.Q. The squares are observations on Fitzgerald, the circles observations on O'Connor.

If we assume that the oxygen consumption due to the protein remains constant at 15 p.c. of the total oxygen when no carbohydrate is being oxidized, the R.Q. can vary between 0·72 and 0·97. Within this range the change in log (CO_2 tension) and consequently *p*H will be on the average 0·04.

With this demonstration of the relation between R.Q. and *p*H, the main purpose of this communication is finished. It remains, however, to show that the results are quantitatively consistent with the previous observations made on the rabbit. In the rabbit it was shown statistically

that a fall of *p*H of between 0·11 and 0·20 would be necessary to produce isodynamic equivalence on the substitution of carbohydrate for fat, if it be produced in the manner suggested. If allowance is made for the steeper slope of the graph of oxygen against *p*H at *p*H 7·30 a change of 0·08 might be adequate. The change (0·04) here calculated is definitely too small.

As this series of observations was not planned to test the precision with which a constancy of energy production was achievable, it would not be surprising if they did not in fact illustrate the isodynamic law; and if the theory of the significance of *p*H be correct they should in fact not exhibit it, as the change in *p*H is on the average too low.

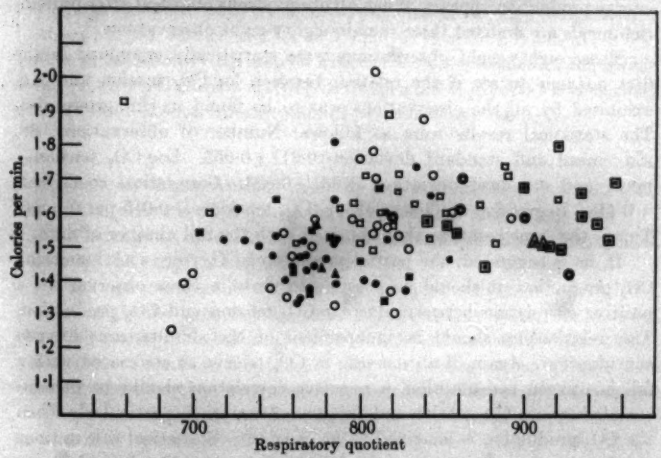

Fig. 2. The relation of calorie consumption to R.Q. Triangles—observations before breakfast; black squares—observations before mid-day; white squares—observations in afternoon on Fitzgerald. Black circles—observations before mid-day; white circles—observations after mid-day on O'Connor. Double contour to an observation indicates the consumption of additional carbohydrate.

Exactly to examine the extent to which the energy production remains constant in these observations, a knowledge of the amount of protein metabolized during each observation would be necessary. An approximate judgment can, however, be made by calculating the energy consumption from the well-known Zuntz table of the energy value of oxygen at the given (non-nitrogenous) R.Q. This is done in Fig. 2, for

the purpose of which the few R.Q.'s below 0·7 have been assumed in calculation to be 0·7. From the figure it appears that there is a decided rise in energy production around R.Q. 0·8. As a consequence of this it is scarcely possible to speak of even an approach to isodynamic equivalence. Inspection, however, shows that this rise is largely due to the inclusion of the observations on Fitzgerald after meals containing meat. An increased metabolism in these observations is to be explained as a specific dynamic effect of protein. Whatever be the nature of the specific dynamic effect, it entails the removal of these data from any comparison on an energy basis with the other determinations. If these observations be left out of consideration in the graph, a closer approach to constancy in energy production appears. When all observations obtained after protein-rich meals are omitted there remain eighty-eight observations.

These eighty-eight observations were statistically examined in the first instance to see if the relation between log CO_2 tension and R.Q. exhibited by all the observations was to be found in this group also. The statistical results were as follows: Number of observations, 88. R.Q., mean and standard deviation, $0{\cdot}811 \pm 0{\cdot}063$. Log CO_2 tension—mean and standard deviation, $1{\cdot}631 \pm 0{\cdot}023$. Correlation coefficient, $+0{\cdot}418$. Regression coefficient of log (CO_2 tension), $+0{\cdot}015$ per 0·1 R.Q. This is the same result as that obtained with the full number of data.

If, as is suggested, the partial pressure of CO_2 rises with increasing CO_2 production, it should be possible to show in these observations a positive correlation between alveolar CO_2 tension and CO_2 production. This relationship should be independent of the simultaneous oxygen consumption. Again, if an increase in CO_2 tension is associated with a fall in oxygen consumption, a negative correlation should be demonstrable between CO_2 tension and oxygen consumption, particularly when the CO_2 production is imagined to be constant. Statistical calculations bearing on these points give the following results: Number of observations, 88. Log (CO_2 tension)—mean and standard deviation, $1{\cdot}631 \pm 0{\cdot}023$ (as above). Carbon dioxide output per min.—mean and standard deviation, 257 ± 30 c.c. Oxygen consumption per min.—mean and standard deviation, 316 ± 26 c.c. Correlation coefficients,

$$r_{12} = +0{\cdot}396;\ r_{13} = +0{\cdot}159;\ r_{23} = +0{\cdot}795.$$

The first of these shows that a rise of CO_2 tension is associated with a rise of CO_2 production, the second shows a positive correlation between CO_2 tension and oxygen consumption, which is too small to be significant and might be due to the close positive association between the carbon

dioxide production and oxygen consumption indicated in r_{23}. That this is so is shown by the partial correlation coefficients which are as follows: $r_{12\cdot3} = +0{\cdot}45$; $r_{13\cdot2} = -0{\cdot}28$; $r_{23\cdot1} = +0{\cdot}86$. From the second of these it appears that when the CO_2 production is imagined constant, an increase in log (CO_2 tension) is associated with a fall in oxygen consumption. The probability of the correlation coefficient being a chance occurrence is, according to Fisher's [1930] table, 0·01, so that the relation may be regarded as established. The most probable quantitative relation between oxygen consumption and CO_2 tension, as calculated from the partial regression coefficient and the corresponding partial standard deviations, is a fall of 18 c.c. for a rise of 0·10 log (CO_2 tension) around the mean oxygen consumption of 316 c.c. Equating change in $-\log$ (CO_2 tension) to change in *p*H, this gives a fall of 5·5 p.c. oxygen for a fall in *p*H of 0·1. This is in satisfactory agreement with the results obtained in the rabbit at 36° [Kane & O'Connor, 1937], in which a fall in *p*H of 0·1 is associated with a fall of oxygen consumption of between 3 and 5·8 p.c.

Summary

In a series of observations taken on two individuals it is shown that the partial pressure of CO_2 in the alveolar air rises with rising R.Q.

The change in alveolar CO_2 tension corresponds to a fall in *p*H of 0·04 over the range of R.Q. 0·72–0·97.

When observations in which a marked specific dynamic effect from protein might be expected are omitted, the energy consumption with changing R.Q. approaches uniformity. Statistical analysis of these observations shows that the CO_2 tension rises with rising carbon dioxide production, and that the oxygen consumption falls with rising CO_2 tension.

The probable extent of the influence of CO_2 tension on oxygen consumption is quantitatively comparable with the influence of *p*H on oxygen consumption in the rabbit.

The relation of these observations to the isodynamic law is discussed.

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SYNCHRONIZED REACTIONS IN THE OPTIC GANGLION OF *DYTISCUS*

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The electrical activity of the optic ganglion of the Water Beetle (*Dytiscus marginalis*) was studied some years ago and the main results were summarized in a note [Adrian, 1932]. One feature of particular interest was the occurrence of rhythmic potential oscillations in the ganglion and of rhythmic bursts of impulses in the nerve, due apparently to synchronous activity in the nerve cells. The tendency to synchronization is now recognized to play a considerable part in the reactions of the cerebral cortex, and it has become important to know more about the conditions which promote it. The optic ganglion of *Dytiscus* is in some ways an ideal preparation for a study of this kind. It has a relatively complex structure—indeed according to Zawarzin [1914] it is equivalent to the retina and optic lobes of a vertebrate—but its electrical reactions are simple and there is the great advantage that its activity can be readily controlled. It has therefore been re-examined in the hope that the results would show what might be expected of nerve cells in general. Although they illustrate a principle which has been invoked before, many of the results can be simply explained and they suggest an explanation for some of the reactions of the cerebral cortex.

Structure of the ganglion

The ganglion is a small conical mass with its base applied to the compound eye and its apex forming the optic nerve which runs immediately into the supra-œsophageal ganglion on that side (Fig. 1). A section through the median plane of the ganglion shows the nerve cells forming an outer layer surrounding a central spherical mass of dendrites.

A similar mass forms a curved plate near the base of the cone. The optic nerve fibres come from the central mass, and the whole arrangement of cells, dendrites and axons probably resembles that of the optic ganglion of the Dragon fly (*Aeschna*) which has been described by Zawarzin [1914].

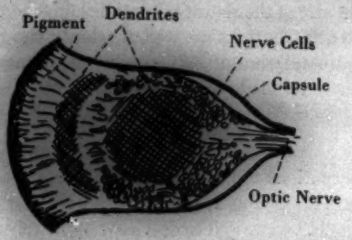

Fig. 1. Diagram to show arrangement of nerve cells and dendrite layers in the optic ganglion of *Dytiscus*.

Method of preparation

The preparation is made by decapitating the beetle, removing the chitin which forms the roof of the head, and clearing away the muscles, tracheal tubes, etc., to expose the paired supra-œsophageal and lateral optic ganglion. The supra-œsophageal ganglion on one side is separated from its fellow and from all other structures but the optic nerve. It is then used as a handle for the optic nerve, and is lifted on to a small ring of silver or platinum wire which forms one of the recording electrodes. The wire is carried in a glass tube fixed in a block of plasticene so as to allow its position to be adjusted. The other electrode is a similar glass handled wire touching the optic ganglion or the tissues near it in the front of the head. Three or four electrodes of the same kind are sometimes used for making simultaneous records from different parts of the ganglion. Non-polarizable electrodes with the metal parts shielded from the light have been used occasionally to make sure that the results have not been due to photoelectric currents from the electrodes. The preparation is set up in a metal box lined with wet cloth, and is irrigated occasionally with Ringer's fluid. As a rule the optic nerve fibres do not survive as well as the ganglion, since they are more liable to damage from drying, but optic nerve impulses can usually be recorded for 3 or 4 hours, and a ganglion has shown typical nervous activity as long as 36 hours after it was set up.

Recording system

The potential changes were amplified with a direct-coupled or condenser-coupled amplifier leading to a Matthews' oscillograph. When the nerve impulses were to be recorded, small coupling condensers were often used to reduce the size of the slower potential changes due to the ganglion, for when the potential rhythms are well developed the ganglion may produce a sinusoidal oscillation of half a millivolt or more. Simultaneous records were sometimes made with two or three oscillographs, used with balanced input amplifiers.

Illumination

Between observations the eye was kept in complete darkness in the preparation box. As the room was darkened for the oscillographic recording the eye could be exposed to dim light by merely raising the velvet curtain covering the front of the box. For very bright light a small 18 watt electric lamp with a metal reflector was set at distances ranging from 24 to 2 in. Intermediate degrees of illumination were given by an opal screen lit by a lamp with a variable resistance to control it, or by altering the general lighting of the room. This was usually so dark that raising the curtain admitted scarcely enough light to make the eye visible to an observer looking into the box, but the illumination as given by a photoelectric cell was generally about $\frac{1}{10}$ m. candle or more. The illumination given by the lamp 2 in. from the eye was about 50,000 times as great. No attempt was made to estimate the exact light intensity or to mark the exact moment of illumination. In the records a hand-operated signal indicates the periods of light and darkness, but it is not intended to give a measure of the latency of the response.

Results

In the fresh uninjured preparation there is no sign of a co-ordinated rhythm except under very bright light. Within 3 or 4 hours a slow potential oscillation appears when the eye is in complete darkness and disappears whenever it is illuminated. The abolition of this "dark rhythm" by light is one of the phenomena with which we shall be concerned, but it must be recognized that the rhythm is to some extent an abnormal event. Its appearance is probably connected with the death of some of the nerve elements from drying, for it can be produced in the fresh preparation by injuring the ganglion. Before the dark rhythm is considered, therefore, the response of the intact ganglion and nerve will be described.

Reactions of the fresh preparation

When the fresh preparation is in complete darkness, the record usually shows occasional spikes indicating an irregular discharge of small impulses at a low frequency in the optic nerve and an irregular oscillation, indicating some electrical activity in the ganglion. The supra-œsophageal ganglion, if attached to the optic nerve, does not contribute to this activity, for it may be crushed or removed without affecting the record appreciably.

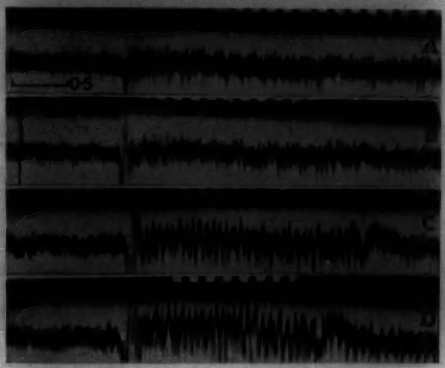

Fig. 2. Discharge of impulses in the optic nerve in response to different illuminations. Fresh preparation. Condenser-coupled amplifier with short time constant. A, very feeble light, raising curtain of box, room dark. B, feeble light, raising curtain, room lighter. C, lamp at 18 in. D, lamp at 3 in. Discharge at 20 per sec. and rhythmic after-discharge at 14 sec. Time: 0·5 sec. marked on record.

On exposure to the feeblest light (by raising the curtain a little way) there is an immediate increase in the impulse discharge. The records in Fig. 2 show the effect of different intensities of light: in all of them the frequency is greatest at the moment of illumination, but there is evidently a progressive increase in the discharge with increasing light. Unfortunately it has not been possible to record from single nerve fibres; with very bright light however (D), there is a regular series at 20 per sec. after the initial peak is over. With moderate illumination (B) the discharge shows no regularity, and is what might be expected from any collection of receptors acting independently of one another. The impulses in each fibre probably form a regular series, though the total response is irregular.

In a few preparations after exposure to medium illumination there has been a slight and momentary increase in the discharge when darkness is restored. More often there is no "off" discharge; the irregular discharge of small impulses continues in the dark but does not vary in frequency.

The first sign of a generalized rhythm appears when the eye is very brightly lit, e.g. by an 18 watt lamp a few inches away. The discharge

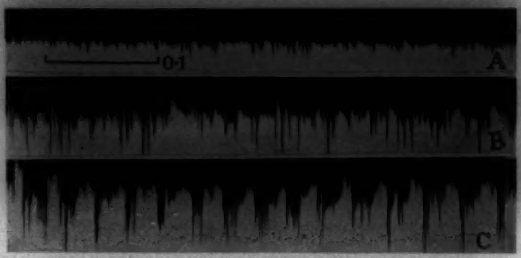

Fig. 3. Grouping of impulses on exposure to very bright light. A, darkness. B, lamp at 18 in., irregular response. C, lamp 2 in. from eye. Initial frequency of groups 36 per sec. Amplifier with short time constant. A downward movement of the base line indicates negativity of the ganglion relative to the nerve. Time: 0·1 sec. marked on record.

Fig. 4. Development of bright rhythm on exposure to very bright light. Waves at 25 per sec. falling to 17 after 1 sec. exposure. A further sudden slowing of the rhythm can be seen when the light is cut off. Time: 0·1 sec. marked on record.

then begins as usual with a crowded irregular succession of impulses, but within a second or less the impulse spikes tend to fall into definite groups recurring with a frequency of between 20 and 40 per sec. as in Figs. 3 and 4—and at the same time a corresponding potential oscillation develops in the ganglion. This effect will be referred to in future as the "bright rhythm". If the lighting is continued the frequency declines slowly, reaching a value of 15–25 per sec. within 5 sec.; if the illumination is reduced the rhythm occasionally continues at a slightly lower frequency, but usually the regular potential waves and impulse groups disappear leaving an irregular discharge.

In the fresh preparation, therefore, a regular response appears only with intense excitation, and ceases when the excitation is diminished. There is no generalized rhythm with illuminations which would be normal for the beetle under water, and none when the eye is in darkness. This frequency of the bright rhythm is not invariable, since it declines with time, but as a rule it cannot be varied by altering the illumination.

Later reactions

If the preparation has been 2–3 hours in the moist chamber the bright rhythm often shows more flexibility. There is still no rhythm in the dark or with moderate light, but the frequency of the rhythm with bright light

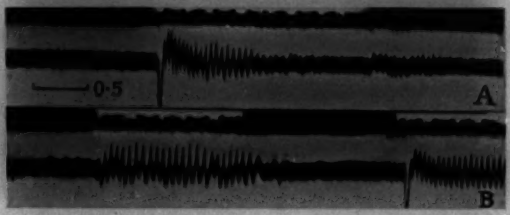

Fig. 5. An example of a bright rhythm showing some variation with the intensity of the light. A, lamp at 2 in. Initial frequency 28 per sec. Rapid fall to 20 followed by disappearance of waves. B, first exposure, to lamp at 18 in. Frequency 20 per sec. Second exposure, to lamp at 6 in. Initial frequency 24 per sec. Time: 0·5 sec. marked on record.

can often be varied over a small range by a change of illumination. Thus, in one experiment when the bright rhythm had fallen to 18 per sec., it could be reduced to 16 by moving the light farther away and restored to 18 by bringing it back. In another, illustrated in Fig. 5, a very bright light gives a rhythm of 28 per sec. with a rapid failure, and a less bright light gave a longer discharge with a rhythm of only 20 per sec. Besides the greater variability in the bright rhythm, there is often a tendency for the waves to persist for a short time after the light is cut off. During these rhythmic after-discharges the frequency falls rapidly, sometimes to values as low as 8–10 per sec.

The dark rhythm

Sooner or later (sooner if the preparation is not kept moist) a rhythmic potential change appears when the eye is in complete darkness, disappearing whenever it is feebly lit. The dark rhythm usually starts as a sequel to the bright rhythm, the frequency falling to 7–9 per sec. when

the light is turned off. In fact in its earlier stages the dark rhythm might be considered an after-discharge which tends to perpetuate itself. To produce it the eye must be adequately stimulated. Thus, in Fig. 6, a brief exposure to bright light gives a short after-discharge at a frequency of about 14 per sec. A longer exposure gives an after-discharge with larger waves and a slower decline, and with a still longer exposure the waves continue at a steady rhythm of 12 per sec. as though the level of excitation could now maintain itself at a fixed value.

In the next stage the slow dark rhythm, once started, will persist, but once abolished will not reappear except as a sequel to the bright rhythm.

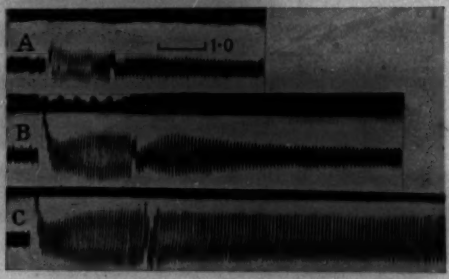

Fig. 6. Development of the dark rhythm as a persistent after-discharge. A, short illumination, short after-discharge of small waves. B, longer illumination (same intensity), after-discharge lasting 5 sec. C, still longer illumination. After-discharge continues indefinitely. The frequency has fallen to 12 per sec. at the end of the record. Time: 1·0 sec. marked on record.

Eventually, however, the waves return whenever the eye is restored to complete darkness, and may persist for several hours with little change in frequency and an amplitude as large as 1 mV. The rhythm sometimes disappears if the eye is kept continuously in the dark for a long period, but brief illumination followed by darkening will restore it, usually to a frequency which is relatively high and declines slowly to the steady value. Thus occasional periods of illumination seem to be necessary both to start the rhythm and to maintain it, as though without them the activity tends to fall below the level required for a regular discharge.

Although the preparation may remain active for many hours after the dark rhythm has appeared, there can be little doubt that it is a sign of some pathological change. If there has been any damage in the dissection the rhythm may appear at once, and it can be produced at any time by

cutting into the optic ganglion at the point where the nerve leaves it. Cutting the nerve itself seems to have less effect than damage to the ganglion, though the damage need not be extensive and may be confined to the apical part. In some preparations puncturing the capsule has been enough to start the dark rhythm. That injury should favour synchronization is not surprising, for it is known to do so in peripheral nerves. In this case, however, it is difficult to say how it operates, as we do not know what particular structures have to be damaged.

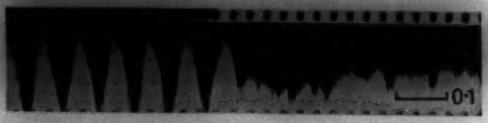

Fig. 7. The dark rhythm. Potential waves at 12·5 per sec. abolished by exposure to faint light. In this record the amplitude of the waves is about 80μV.: they are often as large as 500 μV. Time: 0·1 sec. marked on record.

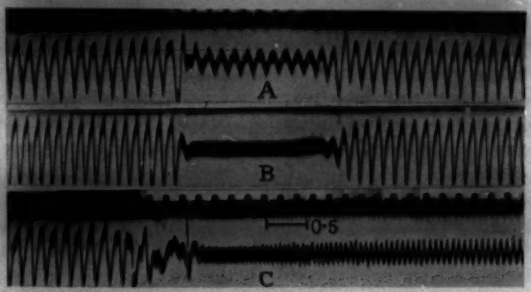

Fig. 8. The dark rhythm is reduced or abolished by light. If the light is intense the bright rhythm appears. A, dark rhythm, 7 per sec. Waves diminished by very faint light (raising curtain slightly). B, waves abolished by faint light (raising curtain fully, room dark). C, appearance of bright rhythm. Lamp at 4 in. abolishes the dark rhythm and produces a bright rhythm of initial frequency 20–18 per sec., falling to 14 per sec. Time: 0·5 sec. marked on record.

Absence of rhythm in dim light

When the dark rhythm has appeared there is a regular potential oscillation at 7–10 per sec. in complete darkness as well as that at 20–30 per sec. in bright light. But exposure to dim or medium light always abolishes the large potential waves of the dark rhythm and restores a steady base line. Figs. 7 and 8 show the partial and complete abolition

of the dark rhythm by dim light, as well as the development of the bright rhythm by intense light. There is sometimes a slight fall in the frequency of the waves when they are reduced, but the main effect is the change in size (Fig. 16). The amount of light needed is very small—raising the curtain of the preparation box is enough—and, except with the very feeblest light, there is no return of the dark rhythm until the curtain is lowered again.

Thus the ganglion will now give the two rhythms, in complete darkness and in very bright light, but in dim or medium light there is no regular response. Both in the bright and the dark rhythm the frequency may show a progressive decline from its initial value, but the rhythms are fixed in the sense that they cannot be altered (except over a small range for the bright rhythm) by altering the illumination of the eye.

Analysis of results

The nature of the two response rhythms

It is evident that the rhythmic potential changes must be due to synchronized action in a large number of units. It does not follow that all the neurones tend to become active and inactive simultaneously; in fact it will appear later that the active region may be conducted from one part of the ganglion to another during each wave. None the less, for such regular oscillations there must be a very close co-ordination between the different neurones. Thus a failure of the waves might result from a failure of co-ordination as well as from a failure of activity. The question which then arises is why the ganglion tends to give only the two fixed rhythms which have been labelled "bright" and "dark". Why does feeble illumination suppress the waves of the dark rhythm and give none of its own in return? The problem is important, because in other groups of neurones we find the same tendency to give definite potential rhythms which appear and disappear but do not change appreciably in frequency. Berger's α rhythm in the human cortex is a good example; indeed the abolition of the α waves in man has already been compared with that of the dark rhythm in the water beetle [Adrian & Matthews, 1934 *b*]. An analysis of the optic ganglion response may help to decide whether these fixed rhythms are merely those at which synchronization can occur, or whether they are due to certain neurones which can only respond at a fixed rate. The latter possibility is unlikely, for in sensory endings and motor neurones the response frequency can vary over a wide range, but there are some kinds of active cell in which it varies very little.

The fixed rhythms in the optic ganglion can be simply explained on the first supposition, but not on the second. It is reasonable to assume that the neurones will be unable to develop a synchronous beat unless they are all excited to much the same degree, and so are all tending to beat at much the same frequency. With a very bright light they would be excited to their maximum rate, and it will need very little adjustment to make the periods of discharge coincide in all of them. Thus the bright rhythm can be obtained even in the fresh preparation. If it can be assumed that both the bright and the dark rhythm are due to the same neurones the latter might occur because in complete darkness there will be no external stimulation to determine the frequency in each neurone: some will give a spontaneous "resting" discharge at a low rate, probably more will do so if the preparation is injured and they will be more able to influence their neighbours. Thus in the dark there will be nothing to prevent the neurones beating together at a slow rate corresponding to the resting discharge. In feeble or moderate light, however, the neurones will be excited to very different degrees, since the thresholds for the different receptors in the eye will vary and so will the amount of light falling on them. Thus the frequencies will differ too widely to allow synchronization.

This effect of feeble illumination is illustrated in Fig. 9 which gives three imaginary intensity-frequency curves of the usual exponential form. The curves are all alike, but the first is supposed to apply to a receptor which needs one light unit for threshold excitation, the second needs two units and the third three. It is assumed that a tenfold increase of light intensity covers the whole frequency range, whereas in reality we are dealing with intensities varying from 1 to 10,000 or more, but the form of the curve is all that matters and this agrees with Hartline & Graham's [1932] measurements on the eye of *Limulus* and with the curves from other receptors. To show the effect of dim and of bright light two vertical lines have been drawn corresponding to illumination of 4 and 16 units. With the dim light the frequencies of response in the three receptors are 10, 14 and 24 per sec. With the bright light they are 26·5, 29 and 30. Thus with the dim light the frequency of two of the receptors would have to be changed by about 40 p.c. to produce a synchronous beat. With the bright light a change of less than 10 p.c. would bring all three to the same value.

Evidently with very bright light a synchronous beat could develop even though the neighbouring units could only exert a slight effect on one another. A much greater degree of interaction would be needed to synchronize the responses to dim or medium light; and if it existed the

eye would be valueless for pattern vision since it would no longer be possible for differences in intensity to be signalled by different frequencies of discharge in the various nerve fibres. In darkness the different units, if isolated from one another, might give resting discharges at widely different rates, but a slight degree of interaction could bring them into line, since there is no external stimulus to set the pace.

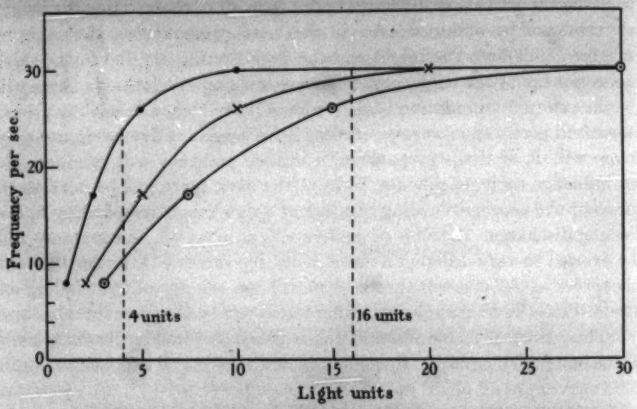

Fig. 9. Imaginary curves relating stimulus (light units) and frequency of discharge for three receptors of different threshold. In faint light (4 units) the frequencies differ much more than in bright light (16 units).

If this explanation of the two rhythms is to be accepted, we must be satisfied that the same neurones produce both slow and rapid rhythms and can respond at intermediate frequencies as well. This would at once rule out the alternative hypothesis of neurones with a fixed rate of response. It will be shown that intermediate frequencies can certainly occur in conditions which would favour synchronization. It is possible that the persistent dark rhythm has a different explanation, but this does not affect the general argument.

Intermediate frequencies

It has already been stated that if bright light is followed by complete darkness the bright rhythm may slow down gradually until the frequency is that of the dark rhythm (cf. Fig. 10). A gradual slowing does not happen in every preparation but it has happened often enough to make it improbable that the two rhythms are due to two completely independent

sets of neurones. Moreover, when there is an abrupt fall from the high rate to the low there is no definite interruption in the sequence of waves. This is illustrated in Fig. 4.

With a preparation which gives the gradual slowing when the light is turned off, an interesting variation is obtained when bright light is

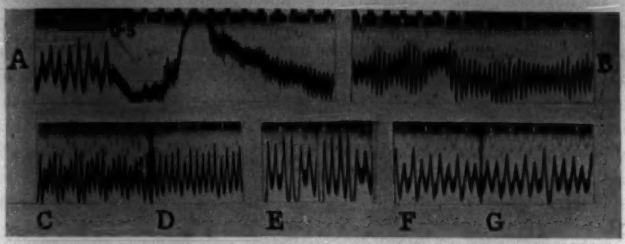

Fig. 10. Gradual slowing of the rhythm when bright light is followed by darkness. A, dark rhythm, 10 per sec. Light on. Bright rhythm, 24 per sec. B, light on 5 sec. Bright rhythm, 18 per sec. Light off. Complete darkness. C, 7 sec. after B. Rhythm 16 per sec. D, 12 sec. after. 14 per sec. E, 19 sec. after. 11 per sec. F, 26 sec. after. 10 per sec. G, 35 sec. after. 9 per sec. Time: 0·5 sec. marked on record.

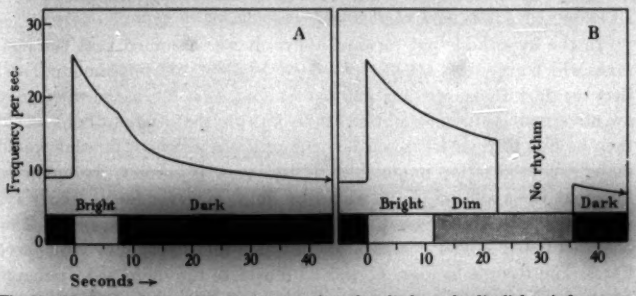

Fig. 11. Abolition of the middle frequencies of an after-discharge by dim light. A, frequency of potential waves when bright light is followed by complete darkness. B, frequency when bright light is followed by dim light and then by complete darkness.

followed by dim light. The rhythm declines until it reaches a value well above that of the dark rhythm, and the waves then cease abruptly. The dimmer the light the lower is the frequency to which the rhythm will fall before the waves disappear. Complete darkness restores them, usually at a lower frequency, and the decline then continues until the normal dark value is reached. The result is shown in the curves in Fig. 11.

On the hypothesis outlined above it can be explained without difficulty as follows. In the preparations in which there is a gradually declining after-discharge, the change from bright light to complete darkness evidently produces a slow instead of an abrupt fall in the level of excitation. The synchronous beat can continue as the excitation declines because, in the darkness, there is no external stimulation to impose different rates on the different neurones. But if bright light is followed by dim light, the waves can only persist as long as the uniform excitation left by the bright light exceeds the non-uniform excitation produced by the dim light. As soon as the frequency has fallen below the average value imposed by the dim light, the inequalities of excitation will assert themselves and will abolish the rhythm; and if the light is moderately bright the rhythm will not decline much from its maximum value before it is broken up.

Thus if there is no chance of uneven excitation, we can often obtain synchronized rhythms over the whole frequency range from 6 to 40 per sec. But with dim or medium light the excitation is bound to be uneven: consequently, the middle range of frequencies can only be obtained as an after-effect of the bright rhythm. It is clear, however, that the neurones can respond over a wide range and are not restricted to one or two fixed rhythms.

The dark rhythm and its abolition by light

In the hypothesis put forward above, it was assumed that the neurones which give rise to the potential waves are all of the same kind, and that the dark rhythm represents their spontaneous discharge when they are not stimulated. The abolition of the dark rhythm by faint light would then be due to their increased but non-uniform activity, for this would make synchronization impossible. If this view is correct, we ought to find that the abolition of the waves by light, however feeble, is always associated with an increased impulse discharge in the optic nerve. This has been found in every preparation in which there were surviving nerve fibres. The records in Figs. 12 and 13 illustrate the increased discharge associated with the failure of the rhythm—they were made with small coupling condensers to show the impulses more clearly. It can be seen also that when either the dark or the bright rhythm is present the impulses are discharged in groups more or less in phase with the potential waves, whereas in dim light the grouping is lost and the waves abolished.

There is, however, another possibility which cannot be completely ruled out, namely, that the persistent dark rhythm is due to a distinct group of neurones which are only active when the eye is in darkness. Hartline [1937] has found that the eye of *Pecten* contains receptors

which are stimulated by the change from light to darkness, and may continue in action for long periods in the dark. The vertebrate eye also shows an increased discharge in the optic nerve when the light is cut off [Adrian & R. Matthews, 1928], and Hartline finds that the nerve fibres in which the "off" discharge takes place are not the same as those which are active when the eye is illuminated [Hartline, 1935]. In some *Dytiscus* preparations a small "off" discharge has appeared when the

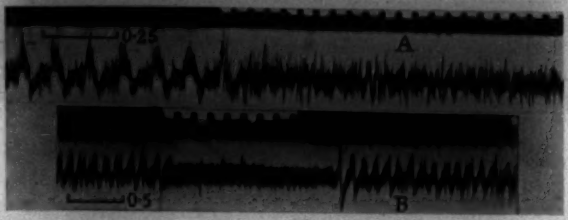

Fig. 12. Increased discharge of impulses when the dark rhythm is abolished by dim light. Amplifier with short time constant to reduce the size of the slow waves. A, dark rhythm at 9 per sec. Faint light (raising curtain) gives an irregular discharge of impulses. B, another preparation. Dark rhythm at 10 per sec. with impulse groups at the same frequency. Faint light gives a rapid discharge. Time: 0·25 and 0·5 sec. marked on records.

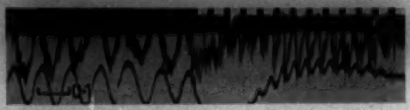

Fig. 13. Exposure to bright light abolishes the dark rhythm, giving an impulse discharge which becomes grouped as the bright rhythm developes (at 24 per sec.). Upper tracing made with amplifier with short time constant to show impulses, lower with amplifier with long time constant to show waves. Time: 0·1 sec. marked on record.

previous exposure to light was carefully adjusted: in most there is no increase in activity when the light is cut off, but there is always a persistent irregular discharge of small impulses whilst the eye is in darkness (cf. Fig. 3). Presumably the dark rhythm is obtained when the neurones which give these small impulses become synchronized; and as the impulses produced by illumination are on the whole larger, it is evident that there are some neurones which contribute to the bright rhythm but not to the dark. But the continuous decline in frequency which may occur on darkening shows that the neurones responsible for the

bright and dark rhythms cannot be completely independent. In records of the impulses accompanying a rhythmic after-discharge it is found that the groups which appear with each wave are made up of spikes of all sizes: as the frequency of the waves declines the number of large spikes in each group becomes less, and as the final value of the dark rhythm is approached the large spikes are found only occasionally. It is possible that the large spikes are due, not to larger nerve fibres, but to several acting in unison; but in any case it is only at the lowest frequencies that they are completely absent. The arguments as to synchronization can therefore be applied to all but the lowest rhythms, and may be applicable to these also. If the persistent dark rhythm is due to special receptors and needs stimulation by darkness to maintain it, the abolition

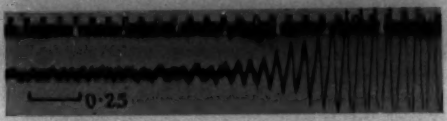

Fig. 14. Sudden development of a bright rhythm at 16 per sec., 4 sec. after the beginning of illumination. Time: 0·25 sec. marked on record.

Fig. 15. Progressive return of the dark rhythm (12 per sec.) after its abolition by light. Time: 0·5 sec. marked on record.

of the waves by light must be attributed merely to the withdrawal of the stimulus. But where a rapid rhythm has been present during illumination, has declined gradually when the light is cut off and is then abolished by light of medium intensity, it is more likely that the waves fail owing to desynchronization than owing to the absence of a dark stimulus.

There are other lines of evidence to support the view that the conditions in which the rhythms appear are merely those in which synchronization is possible. One point is that the waves often appear and increase rapidly in size some time after the appropriate conditions have been established. In Fig. 14, for instance, the waves of the bright rhythm develop in this way without any change in frequency. Fig. 15 shows the progressive return of a dark rhythm after its abolition by light, and both figures recall the rapid, progressive development of large potential waves

in injured nerve trunks and other collections of excitable cells which can respond synchronously [cf. Adrian, 1930; Adrian & Gelfan, 1933; Hoagland, 1933].

During the abolition of the dark rhythm by dim light there is sometimes a slight but distinct fall in frequency (Fig. 16). This certainly

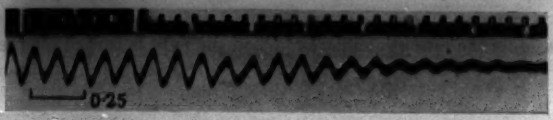

Fig. 16. Dark rhythm reduced in amplitude by gradually increasing light. The frequency falls from 10 per sec. to 9. Time: 0·25 sec. marked on record.

favours the view that the rhythm is abolished because the dark stimulus has failed; on the other hand it might result from the elimination of the more excitable neurones which would set the pace of the resting discharge, but would cease to control it if their frequency was increased by the light.

As we might expect, when the waves of the dark rhythm are reduced in size by light, although there may be little change in frequency there is

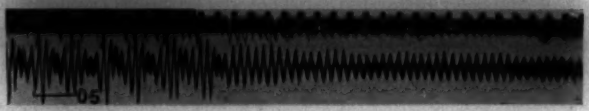

Fig. 17. Complex dark rhythm. Exposure to faint light eliminates one component. Time: 0·5 sec. marked on record.

evidently a reduction in the number of neurones which contribute to the synchronized waves. This can often be seen when the waves are complex, for a very feeble light may then simplify them by eliminating one of the components (Fig. 17).

Summation of potentials in the ganglion

The increased discharge of impulses in the nerve shows that on the whole the activity of the ganglion is increased by feeble light, although the large waves are abolished. Further evidence of the increased activity comes from records of the steady potentials which are developed on exposure to light. When these are made (with a direct-coupled instead of a condenser-coupled amplifier) they show a sustained negativity of the ganglion,

increasing with the light; there is no sign of any reversal of the effect associated with the failure of the dark rhythm.

A typical record is given in Fig. 18 A. The dark rhythm is present at the beginning. When the eye is brightly illuminated the ganglion develops a negative potential with respect to the nerve, and it is on this new base line that the waves of the bright rhythm appear a third of a second later. With continued illumination the potential difference declines gradually, and so does the rate of oscillation. When the light is turned off the base line returns to its original position, the dark rhythm appearing later (not

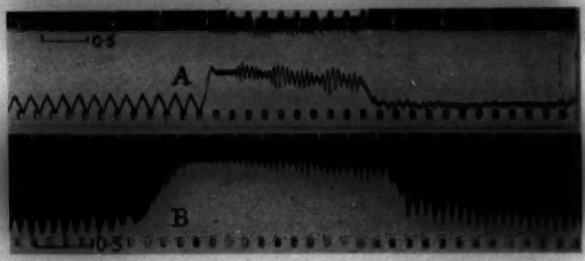

Fig. 18. Records with battery-coupled amplifier showing the persistent negativity of the ganglion on exposure to light. With this amplifier the base line moves upwards when the ganglion becomes negative to the nerve. A, dark rhythm, 6 per sec. Exposure to lamp at 6 in. makes the ganglion 0·7 mV. negative to the nerve and induces a bright rhythm at 22 per sec. B, another preparation. Dark rhythm, 12 per sec. Exposure to bright light makes the ganglion 0·8 mV. negative and induces a bright rhythm at 22 per sec. After-discharge at 14 per sec. with the ganglion remaining 0·15 mV. negative. Time: 0·5 sec. marked on record.

shown in the figure), but in Fig. 18 B there is both a remainder of the potential shift and a higher frequency than at the beginning. These figures bear a close resemblance to Fröhlich's string-galvanometer records from the cephalopod eye, published 25 years ago [Fröhlich, 1913].

The sustained negativity of the ganglion is found in every preparation. Its magnitude seems to depend on the degree of excitation, for it varies with the nerve discharge when different illuminations are used, tending to a maximum with illuminations strong enough to give the bright rhythm. Also it outlasts the light when there is an after-discharge, but declines abruptly when there is none. That there is a close relation between the potential level and the frequency of the waves can be seen in

Fig. 18, and more clearly in Fig. 19. This was made by moving an electric torch up to and away from the eye. As it is moved nearer the negative potential of the ganglion increases: when the excitation reaches its maximum the neurones begin to work in unison and the waves of the bright rhythm appear, and as the light is moved away the potential declines and the frequency falls from 22 to 15 per sec. before the waves are broken up.

Fig. 19. Battery-coupled amplifier. A bright light is moved towards the eye and then away from it. The ganglion becomes increasingly negative and the bright rhythm appears at 22 per sec. As the light is moved away the potential declines and the rhythm falls to 14 per sec. before the waves fail. Time: 0·5 sec. marked on record.

The close relation between the waves and the steady potential suggests that both may arise in the same neurones. As far as can be discovered by varying the position of the electrodes, they certainly come from the same part of the ganglion. Both the waves and the steady deflexion are greatest when the middle and apical regions are included between the leads, and no arrangement has been found to give greater prominence to one effect rather than the other. But the ganglion is so small that a difference in focus might well have been overlooked.

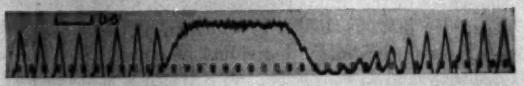

Fig. 20. Battery-coupled amplifier. Dark rhythm (4 per sec.) abolished by faint light. A small negative potential (0·08 mV.) develops in the ganglion. Time: 0·5 sec. marked on record.

Fig. 20 shows that a dim light gives a potential difference in the same sense as a bright light. The illumination may be only just enough to stop the dark rhythm, but invariably the ganglion becomes negative to the distal part of the nerve. The waves in this case were small (less than $\frac{1}{10}$ mV.) for the dark rhythm had only just appeared: later they increased so much that, in comparison with them, the shift in the base line produced by dim light would have been inappreciable, for the potential shift for a given light remains fairly constant whether the dark rhythm has appeared or

not and whatever the size of its waves. The constancy of the steady potential and the variation in the waves fits in very well with the hypotheses that the former is a measure of the total activity and the latter of the degree of synchronization.

Nature of ganglion potentials

As regards the nature of these potential changes, the present observations do not add much to the account given some years ago [Adrian, 1931]. This dealt with the spontaneous activity of the ventral ganglia of *Dytiscus*, where a discharge of impulses along the motor nerve is always associated with a negative potential in the ganglion (relative to the nerve). It was found that the fall of potential began before the nerve discharge and persisted until it was over: from this it was inferred that the activity of the cells and dendrites must involve a relatively slow depolarization, as a result of which one or more impulses are discharged along each axon. The same explanation was suggested for the potential waves in the optic ganglion [Adrian, 1932].

Later work on the potentials in the cerebral cortex [Adrian & Matthews, 1934 *a*] threw some doubt on the possibility of a sustained negative potential in the individual cells, and emphasized the composite nature of many of the slower changes. In the present experiments we are certainly dealing with composite effects: for instance, the persistent negativity when the eye is brightly lit must be due in part to a summation of potential waves in neurones beating out of phase—for the waves are obvious when the neurones come into phase. But however large and regular the waves of the bright rhythm may be, there is always a persistent negativity of the ganglion in addition, and this suggests some persistent depolarization in the individual neurones. Such an analysis would agree with (and was suggested by) Barron & Matthews's [1936] recent findings in connexion with spinal cord potentials: their results are so much more definite, however, that a discussion of probabilities from the *Dytiscus* records would be a waste of time.

Whatever the steady potential may imply, it is clear that the waves are an index of the rhythmic activity of the nerve cells and dendrites. The records in Fig. 21 show the relation between waves and nerve impulses in two records made at a higher speed than usual. The eyes are brightly illuminated, but the bright rhythm has developed to different degrees of regularity. When it is poor (Fig. 21 A) the groups of impulses, though occurring with nearly the same frequency as the waves, are sometimes on the crest and sometimes in the trough, as though they came

from a group of neurones slightly out of phase with the majority. But when the waves are quite regular (Fig. 21 B) the impulses always appear in a fixed relation to them, when the negativity of the ganglion is nearing its maximum.

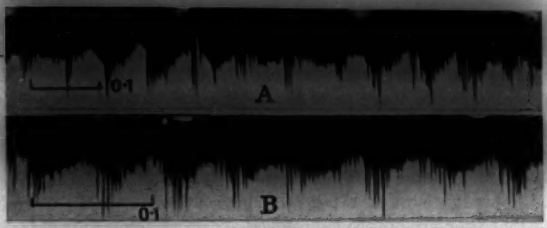

Fig. 21. Relation between waves and nerve impulses during the bright rhythm. Condenser-coupled amplifier with short time constant. The base line moves down when the ganglion becomes negative to the nerve. A, the waves vary in size and the impulses have no constant relation to them. B, another preparation. Regular waves with impulse groups always appearing as the ganglion becomes negative.

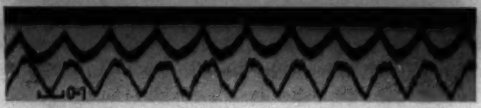

Fig. 22. Simultaneous records of potential change at two pairs of electrodes. Upper tracing, electrodes on nerve and near apex of ganglion. Lower tracing, electrodes near apex and near base. Dark rhythm, 5 per sec. Phase difference between waves in upper and lower tracings, showing movement of active region during each wave. Time: 0·1 sec. marked on record.

The form of the potential waves varies with their frequency, and there is often a near approach to a sinusoidal oscillation both with the dark and the bright rhythm. But the form is governed to some extent by movements of the active region in the course of each wave. The existence of such movements can be detected by recording simultaneously from two pairs of electrodes, one on the base and middle of the ganglion and the other on the middle and apex. The maximum potential change seems to arise from the middle and upper third, but there is often a phase difference between the waves in the two records (Fig. 22), indicating a shifting region of activity. Owing to the small size of the ganglion, no attempt has been made to work out an accurate map of the potential distribution from moment to moment; it can only be said that the apparent wave

form does not necessarily show the rate of rise and fall of activity at a given point any more than the electro-cardiogram shows what is happening at a given point in the heart.

Discussion

This account has been mainly concerned with two abnormal phenomena, the potential oscillations produced by a very bright light and those occurring in the dark when the ganglion has suffered injury. In these conditions the neurones of the optic ganglion react as a mass giving a single rhythm: in conditions nearer the normal for the living animal they react independently, giving a total response in which no rhythm can be seen. Since the eye must be useless for pattern vision when the neurones are working as a single unit, it is scarcely surprising to find that they are only synchronized at the extreme ends of the visual scale—in complete darkness and intense light. But can such abnormal effects tell us anything about the general reactions of nerve cells?

In one respect they confirm what was already fairly clear, namely, that neurones cannot be expected to work synchronously unless the degree of excitation is the same for all of them, so that all tend to beat with the same frequency. This has been pointed out in explanation of the definite 50–80 per sec. rhythm in an intense motor discharge, for the motor neurones are then excited to the maximal degree [Adrian & Bronk, 1929]. It has also been used to explain the 10 per sec. potential waves in the cerebral cortex (Berger's α rhythm), the assumption being that the neurones are unstimulated and are beating at the minimal rate [Adrian & Matthews, 1934*b*]. In the *Dytiscus* ganglion, however, we have both a rapid and a slow rhythm and the results show that these are quite compatible with a response over the entire frequency range in the individual units. This is the main outcome, for in face of it there is no need to suppose that the fixed potential rhythms of the cerebral cortex must imply any inflexible response in the cortical neurones.

When we come to the factors which prohibit intermediate rhythms, it may be more risky to generalize from the *Dytiscus* results. In a discussion of the rhythmic potentials from the vertebrate retina [Adrian & R. Matthews, 1928], a uniform illumination of the visual field was considered necessary for synchronization: the present results emphasize the fact that what is essential is uniform excitation of the neurones rather than a uniform stimulus. Uniform excitation is not likely to be achieved by anything but a very bright light or no light at all, for it is unlikely that all the receptors will have the same threshold and so will give the same

frequency when they are equally lit. The light used by Adrian & R. Matthews to produce a rhythm in the vertebrate retina would certainly rank as bright, and in view of the present results it is probable that a uniform field, if dimly lit, would not give a synchronized response. The distinction between uniform stimulation and uniform excitation is worth drawing, for it may apply to other structures than sense organs. For instance, uniform stimulation of a cortical area might break up a threshold rhythm by exciting the neurones to different levels.

The dark rhythm in the *Dytiscus* ganglion needs abnormal conditions to develop at all and breaks down as soon as any light falls on the eye. If the failure is due to increased but asynchronous activity, it implies a very loose linkage between the different neurones, one which allows them to act quite independently when they are stimulated. This kind of association would be secured if the active period in one neurone were able to exert a slight stimulating effect on its neighbours, for this would ultimately bring all the neurones into line if there were nothing else to affect their responses. It is interesting to find that the association is favoured by injury, for there are several examples of synchronized discharges in nerve trunks which have been injured (e.g. the phrenic [Adrian, 1930]; the lateral line nerve [Hoagland, 1933]). In these there is some reason to believe that the interaction is due to electrical stimulation of one fibre by another, the injury having caused a breakdown of the normal insulation. There are many parts of the vertebrate central nervous system where injury favours interaction as it does in the *Dytiscus* ganglion, but to account for the synchronization at high frequencies we must suppose that in both some degree of interaction must be perfectly normal. In abnormal conditions (e.g. convulsant drugs, asphyxia, repeated stimulation) the degree of interaction in the vertebrate central nervous system may become so much exaggerated that the neurones can no longer work independently. In complex systems, however, the factors which promote a synchronized response may differ to some extent from those considered here; for instance, a single pace-maker in some part of the brain stem might impose its rhythm on a distant group of neurones, which would then react in unison as they would in response to rhythmic stimulation. Where this occurs there should be no restriction to particular frequencies: indeed we may conclude that the restriction of potential waves to a fixed frequency is evidence that the neurones are not very closely linked and are capable of acting independently at different frequencies.

Conclusions

The electric responses of the optic ganglion and nerve of the Water Beetle (*Dytiscus marginalis*) show that the neurones are often working synchronously, giving rhythmic potential waves and a grouped impulse discharge. The factors which promote or hinder the synchronous response have been investigated in the hope that the results might apply to other collections of nerve cells. The results are as follows:

1. In a fresh preparation a generalized rhythm is only obtained when the eye is exposed to very bright light. The bright rhythm lies between 20 and 40 per sec., declining in frequency with time. If the light is reduced the potential waves disappear.

2. Some hours after the preparation has been made, a potential rhythm at 7–10 per sec. appears when the eye is in complete darkness. The dark rhythm is an abnormal reaction, and can be brought on by injuring the ganglion. If the eye is exposed to the faintest light the waves disappear.

3. When the dark rhythm has developed, the ganglion shows the two fixed potential rhythms corresponding to bright light and no light. With dim or medium light there is no generalized rhythm, but there is the usual irregular discharge of impulses in the optic nerve.

4. Since bright light may cause a rhythmic after-discharge of declining frequency, it is clear that the two rhythms are not due to two completely independent groups of neurones with fixed rates of response. The neurones can respond over a wide frequency range and the fixed potential rhythms are those at which synchronization can occur.

5. It is shown that in a collection of receptors of differing threshold the frequencies of response will tend to differ widely when the stimulus is of small or medium intensity. Synchronization will then be impossible. When the stimulus is strong the frequency will be maximal in all and synchronization can occur. When there is no stimulus synchronization might occur at a low rate corresponding to the "resting" discharge.

6. The persistent dark rhythm may possibly depend on the stimulation of certain neurones by darkness: its abolition by light would then be due merely to the withdrawal of the stimulus. It is more likely that the rhythm represents a spontaneous discharge in neurones which respond at a higher rate on illumination. The failure of the waves on illumination would then be due to the neurones being stimulated to respond at different frequencies. In darkness a few neurones might give rise to the rhythm, for there would be nothing to prevent the others coming into line with them provided that some interaction can occur.

7. The potential changes in the ganglion resemble those in other groups of nerve cells. The waves coincide with the discharge of impulses in the nerve.

8. From the reactions of the *Dytiscus* ganglion it is clear that fixed potential rhythms (e.g. in the cerebral cortex) need not imply a fixed frequency of response in the neurones which contribute to the waves. Synchronized waves will be most likely to occur when the frequencies are near the maximal or the minimal values for these neurones.

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THE RELATION BETWEEN PLASMA AND CEREBROSPINAL FLUID CALCIUM

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In 1925 we published a study of the blood and cerebrospinal fluid calcium, especially in relation to the tetany of parathyroid deficiency [Cameron & Moorhouse, 1925*a*], and a note on the action of Collip's parathyroid extract on the calcium content of these fluids [Cameron & Moorhouse, 1925*b*]. Certain conclusions expressed in the first paper cannot now be upheld. Our assumption that the non-diffusible calcium of blood plasma is combined in some compound specifically controlled by the parathyroid principle is at variance with current conception of parathyroid function [Thomson & Collip, 1932; Selye, 1932]. Recent accurate studies do not support the view that plasma and serum calcium differ in amount [Streef, 1936]. Evidence is accumulating that the distribution of calcium in plasma is governed by its protein [Loeb, 1926] and phosphate content [Greenburg *et al.* 1935], and to some extent by its *p*H [Smith, 1934].

Twelve years ago methods involving compensation dialysis and ultrafiltration, then in use to measure the diffusible fraction of blood plasma or serum, were not standardized, and their use had led to discordant results. Accepting the view that cerebrospinal fluid could be regarded as an ultrafiltrate of plasma, we suggested that its calcium content could be taken as equal to the diffusible calcium of the plasma. We found that the ratio between serum and cerebrospinal fluid calcium in normal dogs averaged 1 : 0·53. The spinal fluid calcium was but little affected in tetany following thyroparathyroidectomy, which suggested that parathyroid deficiency chiefly affected the non-diffusible calcium of the plasma [1925*a*]. Injection of parathyroid extract into a normal dog led to a definite rise in the spinal fluid calcium [1925*b*].

In the ensuing interval the assumption that the cerebrospinal fluid calcium is a true measure of the diffusible calcium of the plasma has been increasingly challenged. Following a brief review of recent literature we shall present some further experimental evidence. We do not now propose to discuss the thorny problem of the true nature of the cerebrospinal fluid—dialysate or secretion.

Greatly improved physical chemical methods for direct measurement of diffusible calcium of blood serum have given values in fairly good agreement with those of spinal fluid calcium in man and animals with normal blood calcium. Typical figures for the percentage ratio of spinal fluid to serum calcium are, for dogs, 46–53 [Morgulis & Perley, 1930], for normal man 45–55 [Cantarow, 1929], and for dispensary patients 46–51 [Morgulis & Perley, 1930]. Direct determinations of the percentage ratio of diffusible to total calcium in serum have given such figures as 45–55 [Updegraff *et al.* 1926], and 42–58 [Liu, 1927*a*] in man, dogs and cattle. The average of all reliable figures is, in each case, not far from 50 p.c. Simultaneous determinations in dogs and man also agree fairly well [Snell & Wales, 1930; Hertz, 1930; Greenburg *et al.* 1930; McCance & Watchorn, 1930–1; Gregory & Andersch, 1936]. In all cases showing such agreement the blood calcium was within normal limits. It has, however, been shown that cases with increased protein in the spinal fluid must be excluded, since then the calcium is also increased [Merritt & Bauer, 1931].

There is excellent evidence, therefore, that under normal conditions the figures for diffusible calcium of blood plasma and cerebrospinal fluid are approximately equal. But there is also evidence that this equality ceases when plasma calcium is either elevated or depressed, whether through disease or by experiment.

In osteitis fibrosa and multiple myelomatosis, with high values for serum calcium, the diffusible calcium is definitely greater than that of the spinal fluid. Low serum calcium values in nephritic conditions have correspondingly low values for diffusible calcium, but the spinal fluid calcium is only slightly depressed [Herbert, 1933; Watchorn & McCance, 1932; Gregory & Andersch, 1936].

In agreement with our own results it has been found that in dogs in tetany from parathyroid deficiency the spinal fluid calcium only falls slightly [Morgulis & Perley, 1930]; this is also true for man [Merritt & Bauer, 1931]. A few simultaneous determinations in dogs in tetany showed much greater fall in the diffusible calcium of the serum than in that of the fluid [Hertz, 1930]. Measurements on blood alone in such

tetany are not completely concordant, but indicate that the fall in diffusible calcium is at least as great as that in the non-diffusible fraction [Snell & Wales, 1930; Hertz, 1930; Moritz, 1925; Pincus *et al.* 1926; Gunther & Greenburg, 1931].

Following continuous intravenous injection of calcium salts, the increase in cerebrospinal fluid calcium is negligible compared with that in the plasma [Morgulis & Perley, 1930], but such injection apparently affects both diffusible and non-diffusible plasma calcium to like extent, due presumably to further partial combination with protein [Smith & Sternberger, 1932; Benjamin & Hess, 1933]. A marked rise in blood calcium induced by injection of parathormone is accompanied by a negligible rise in spinal fluid calcium [Morgulis & Perley, 1930; Merritt & Bauer, 1931]. Both in man and the dog parathormone induces a proportional increase of diffusible serum calcium [Snell & Wales, 1930; Benjamin & Hess, 1933; Liu, 1927*b*; Greenburg & Gunther, 1932]. A minority of observers report otherwise [Hertz, 1930; Sykes, 1936].

Basis of the experiments now reported. Formation of cerebrospinal fluid involves transference of fluid into a closed system, and in absence of a true circulation movement must be so slow that chemical changes in plasma can only be mirrored slowly in the fluid. If open drainage of the fluid accelerates its production, then changes in composition of the plasma should produce corresponding changes in the fluid more rapidly. Available experimental methods do not permit perfect contrast between closed and open systems, since repeated tapping of fluid from the closed system involves its subsequent formation with a pressure barrier below normal. Nevertheless it seemed of interest to try to ascertain the extent to which open drainage would accelerate changes in spinal fluid calcium. That some changes are accelerated is proved by the work of Fremont-Smith *et al.* [1930], who showed that when, in human patients, water is taken by mouth after subcutaneous injection of vasopressin, the blood becomes hypotonic; with continuous drainage from the canal the hypotonicity is reflected in the spinal fluid within 2 or 3 hours. (No estimations of spinal fluid calcium were made in their experiments.)

Results

Six series of experiments have been carried out on dogs. In series I, dogs 1 and 2, the effect of continuous open drainage was measured in absence of other treatment. The figures for dog 1 in Table I are typical. In Series II, dogs 3–9, gradually increasing concentrations of calcium

chloride solution, rendered approximately isotonic to plasma by addition of sodium chloride, were injected, also with continuous open drainage. Typical results are shown for dogs 4, 6, 8 and 9. When a concentration had been found which produced a definite effect on the spinal fluid calcium, Series III, dogs 10–12, was carried out with a closed spinal fluid system, tapped for fluid from time to time. In Series IV, dogs 13 and 14, parathormone was injected intravenously, and in the remaining two series, subcutaneously and intramuscularly. In Series IV and V there was continuous open drainage, but in Series VI the open drainage was only started after 16 or 17 hours.

All dogs were anæsthetized with morphine and urethane, and subsequently kept under chiefly with urethane. In certain experiments access to the cisterna magna was obtained by dissecting down to the occipito-atlantoid membrane and inserting a tap connexion of the Zimmermann type. With this arrangement the spinal fluid could be tapped from time to time or continuous drainage established at will. In the later experiments with continuous drainage, a needle puncture was made directly into the cisterna.

In all experiments with continuous drainage the times of sampling were determined by the periods required to obtain sufficient spinal fluid for duplicate analyses, i.e. 4·5–5 c.c., if this was possible without too long delay. The rate of flow, however, differed greatly in different animals. The fluid was almost always clear; it was occasionally slightly tinged with blood to a negligible extent (as evidence by unchanged calcium values in certain cases). When there was definite contamination the samples were rejected.

Blood samples were obtained at appropriate times from the femoral artery. Cheymol & Quinquaud [1932] have shown that, in the dog, serum calcium from arterial blood averages from 0·3 to 0·5 mg. per 100 c.c. lower than that from venous blood. Analyses for calcium were carried out by the method of Clark & Collip [1925]. Duplicates were almost always in good agreement, usually differing by not more than 0·2 mg. per 100 c.c.

No two experiments were exactly parallel; each animal presented its own problem. Only two dogs survived more than 24 hours, and usually artificial respiration had to be resorted to after the first 12 hours. Since the maximum effect of the parathormone is not reached for from 15 to 20 hours, there was thus a difficulty in keeping the animals alive long enough to test the effect of a really high blood plasma calcium on the cerebrospinal fluid. We thought that we might be able to lengthen the

TABLE I. Experimental results

Series	Date	Dog no.	Weight kg.	Nature of experiment	Time hours	Treatment	Serum Ca	C.S.F. Ca	C.S.F. vol. c.c.
I	12. ix. 34	1	24	Nothing injected. Continuous open drainage	0	—	11·3	5·3	—
					1·0	—	11·3	6·1	—
					2·0	—	10·7	5·8	—
					3·0	—	11·1	5·5	—
					4·5	—	11·6	5·1	—
					5·5	—	11·1	5·4	—
II	16. x. 34	4	24	1000 c.c. isotonic saline injected between 0 and 1·6 hours, containing 0·0095 p.c. Ca; 900 c.c. injected later	0	Injection started	10·1	5·1	—
					1·0	—	9·0	5·8	—
					1·6	Injection stopped	—	—	—
					3·0	Injection started	9·8	5·6	—
					4·0	Injection stopped	8·1	5·7	—
					5·5	—	9·4	5·7	—
	30. x. 34	6	40	800 c.c. isotonic saline (0·0189 p.c. Ca) injected between 1·5 and 2·5 hours, and 800 c.c. later	0	—	10·9	5·3	—
					1·0	—	10·8	5·0	—
					1·5	Injection started	—	—	—
					2·5	Injection stopped	10·7	5·5	—
					3·6	Injection started	10·5	5·9	—
					4·6	Injection stopped	—	—	—
					5·0	—	10·2	6·2	—
	13. xi. 34	8	19	1600 c.c. isotonic saline containing 0·042 p.c. Ca injected	0	—	10·3	6·3	—
					3·0	Injection started	10·3	6·7	—
					4·5	Injection stopped	19·3	7·4	—
					5·5	—	17·1	9·6	—
	20. xi. 34	9	24	1500 c.c. isotonic saline containing 0·041 p.c. Ca injected	0	—	11·3	5·6	—
					2·0	Injection started	—	5·7	—
					3·3	Injection stopped	—	—	—
					3·5	—	15·9	6·6	—
					5·3	—	13·3	8·6	—
III	27. xi. 34	10	24	Closed system. 1500 c.c. isotonic saline containing 0·041 p.c. Ca injected	0	—	10·4	5·2	—
					1·0	Injection started	10·9	—	—
					2·5	Injection stopped	—	—	—
					2·7	—	13·2	5·5	—
					3·7	—	11·9	5·2	—
					5·7	—	10·5	5·7	—
	4. xii. 34	11	20	Closed system. 1500 c.c. isotonic saline containing 0·043 p.c. Ca injected	0	—	10·4	5·5	—
					1·0	Injection started	—	—	—
					2·5	Injection stopped	15·6	6·1	—
					3·5	—	12·8	5·6	—
					5·5	—	11·1	6·7	—
	11. xii. 34	12	20	Closed system. 1500 c.c. isotonic saline containing 0·043 p.c. Ca injected	0	—	11·1	4·7	—
					1·0	Injection started	—	—	—
					2·5	Injection stopped	15·8	5·2	—
					3·5	—	15·3	5·5	—
					5·5	—	12·7	5·6	—
IV	18. xii. 34	13	18	Continuous open drainage. Parathormone injected intravenously	0	40 units parathormone	11·0	5·5	8·0
					2·3	40 units parathormone	11·4	4·9	11·0
					4·5	20 units parathormone	—	—	—
					6·3	—	12·3	5·4	11·0
					9·4	—	12·8	5·4	10·0
					11·5	—	11·6	5·0	9·0
	15. i. 35	14	22	Continuous open drainage. Parathormone injected intravenously	0	40 units parathormone	—	—	—
					0·5	—	11·3	5·8	7·0
					1·5	—	—	5·6	7·0
					2·0	60 units parathormone	—	—	—
					4·0	50 units parathormone	—	—	—
					4·5	—	12·2	5·5	9·0
					6·0	50 units parathormone	—	—	—
					7·5	50 units parathormone	—	—	—
					8·5	—	11·7	5·5	7·0
					10·0	50 units parathormone	—	—	—
					12·5	—	11·8	5·8	12·0
					17·0	—	—	5·6	8·0
					17·5	—	—	5·3	4·0

TABLE I (*cont.*)

Series	Date	Dog no.	Weight kg.	Nature of experiment	Time hours	Treatment	Serum Ca	c.s.f. Ca	c.s.f vol. c.c.
V	27. iii. 35	15	18	Continuous open drainage. Parathormone injected subcutaneously	0	100 units parathormone	11·5	6·0	—
					1·3	—	—	6·2	—
					5·0	—	14·3	6·3	—
					5·2	30 units parathormone	—	—	—
					7·5	—	12·5	6·1	—
	3. iv. 35	16	20	Continuous open drainage. Parathormone injected subcutaneously	0	50 units parathormone	10·8	5·9	5·8
					2·0	—	—	6·0	10·7
					3·0	50 units parathormone	—	—	—
					5·0	50 units parathormone	—	—	—
					6·0	—	12·5	5·9	9·6
					8·0	50 units parathormone	—	—	—
					10·0	—	13·8	6·9	4·5
					12·0	—	—	7·1	11·0
					15·0	—	14·5	5·5	8·1
					18·0	—	14·4	5·9	11·5
					20·0	—	—	6·1	5·0
					21·0	—	14·7	—	—
					24·0	—	14·7	7·0	12·4
					25·5	—	15·3	6·7	5·2
	30. iv. 35	17	18	Continuous open drainage. Parathormone injected subcutaneously	0	50 units parathormone	12·9	5·4	4·5
					2·0	50 units parathormone	—	5·5	5·5
					5·0	50 units parathormone	—	—	—
					6·7	—	—	5·8	10·5
					7·3	—	15·1	—	—
					10·2	—	13·3	6·0	2·8
					21·0	—	18·3	6·1	2·6
	8. v. 35	18	23	Continuous open drainage. Parathormone injected subcutaneously	0	40 units parathormone	11·7	5·3	5·4
					2·0	60 units parathormone	—	5·1	7·7
					4·0	40 units parathormone	—	5·3	5·8
					6·0	60 units parathormone	—	—	—
					7·0	—	13·4	5·9	6·4
					9·0	—	13·9	6·0	4·2
	31. x. 35	19	27	Continuous open drainage. Parathormone injected subcutaneously	0	100 units parathormone	10·1	5·2	7·5
					1·0	—	—	5·0	8·2
					2·7	—	—	5·4	9·5
					3·0	100 units parathormone	—	—	—
					4·0	—	11·3	6·0	4·4
					6·0	—	—	6·0	4·9
					8·0	—	11·6	5·9	4·3
					10·0	—	—	5·6	5·7
					11·5	—	10·5	—	—
	14. xi. 35	20	29	Continuous open drainage. Parathormone injected subcutaneously	0	100 units parathormone	11·6	5·5	9·4
					1·7	—	—	5·5	7·3
					3·5	50 units parathormone	—	5·5	4·6
					6·3	50 units parathormone	12·9	5·1	5·6
					8·3	—	—	5·3	4·2
					10·8	—	15·6	5·4	4·2
					12·8	—	—	5·4	5·1
					15·3	—	15·0	5·7	4·8
					18·0	—	—	5·6	5·4
					21·0	—	18·0	6·0	5·7
					23·0	—	—	6·0	6·1
					25·0	—	18·1	6·2	7·5
					27·3	—	19·8	6·5	7·5
	19. xii. 35	21	26	Continuous open drainage. Parathormone injected subcutaneously	0	50 units parathormone	12·7	6·3	6·1
					1·0	—	—	6·7	4·5
					3·0	50 units parathormone	—	6·8	7·2
					5·0	50 units parathormone	—	—	—
					6·0	—	14·1	6·9	4·4
					7·0	50 units parathormone	—	—	—
					9·5	—	14·4	(8·0)	2·3
					10·0	—	13·3	—	—
					11·0	—	13·2	7·0	21·0

TABLE I (*cont.*)

Series	Date	Dog no.	Weight kg.	Nature of experiment	Time hours	Treatment	Serum Ca	C.S.F. Ca	C.S.F. vol. c.c.
VI	28. i. 36	22	20	Open drainage only after 16 hours. Parathormone injected subcutaneously	0	100 units parathormone	12·1	5·6	4·7
					3·0	50 units parathormone	—	—	—
					6·0	50 units parathormone	—	—	—
					16·0	Open drainage started	20·0	6·6	6·2
					17·5	—	—	7·4	7·6
					20·0	—	18·8	8·4	6·3
	17. iii. 36	23	22	Open drainage only after 16 hours. Parathormone injected subcutaneously	0	100 units parathormone	13·0	5·4	—
					3·0	50 units parathormone	—	—	—
					6·0	50 units parathormone	—	—	—
					16·0	Open drainage started	19·6	—	—
					18·8	—	19·5	6·5	—
	24. iii. 36	24	19	Open drainage only after 16½ hours. Parathormone injected subcutaneously	0	100 units parathormone	12·1	5·6	4·1
					3·0	50 units parathormone	—	—	—
					6·0	50 units parathormone	—	—	—
					17·0	Open drainage started	16·0	*	3·8
					18·0	—	—	6·3	2·6
					21·0	—	—	6·1	5·1
					23·5	—	13·9	6·4	5·5
	30. iv. 36	25	21	Open drainage only after 17½ hours. Parathormone injected subcutaneously	0	100 units parathormone	11·9	6·1	7·1
					3·0	100 units parathormone	—	—	—
					6·0	100 units parathormone	—	—	—
					17·5	Open drainage started	18·9	6·7	5·8
					19·0	—	—	6·6	6·9
					20·0	—	18·5	—	—
					21·0	—	20·4	6·8	2·6

* Analysis spoiled.

experiments by only establishing open drainage 16 or more hours after initial injection of parathormone, but animals so treated (Series VI) survived no longer.

The results are shown in Table I. Calcium is expressed throughout in mg. per 100 c.c. serum or fluid.

DISCUSSION

In experiments with long-continued open drainage from the cisterna magna, and production of relatively large volumes of cerebrospinal fluid, during the later stages of which the experimental animal is certainly not in a normal condition, there obviously arises the possibility of damage to the tissues where the fluid is being formed, and therefore of production of an abnormal fluid. In some of our longest experiments, however, the fluid remained crystal clear throughout. In the experiment with dog 16, lasting 25·5 hours, six samples of the fluid, selected at random throughout the experimental period, all gave a negative test for protein. The last sample of the fluid from dog 20, in an experiment lasting over 27 hours, when examined microscopically only showed two lymphocyte cells per c.c., a normal number. The production of fluid thus appears to have been normal throughout these experiments. The but slight increase

found in the calcium values is also supporting evidence, since damage to the meninges leads to increased values for the spinal fluid calcium.

During the experiments the serum calcium and cerebrospinal fluid calcium were measured on twenty-four presumably normal dogs. The extreme values found were 10·1 and 13·0 for the former (only four exceeded 12·0), and 4·7 and 6·3 for the latter, the corresponding averages being 11·25 and 5·6 mg. per 100 c.c. The extreme ratios found were 41 and 61 p.c., the average being almost exactly 50 p.c., in good agreement with that found in similar series.

Injection of slightly hypotonic calcium solution (e.g. dog 4, Table I) seemed to produce a fall in serum calcium to a value a little lower than that of the solution injected; the number of experiments of this nature was too few to stress this result.

The results following injection of large volumes of markedly hypertonic calcium chloride prove, as was to be expected, that for similar increases in serum calcium greater changes take place in the cerebrospinal fluid when there is continuous open drainage than when fluid is merely withdrawn from time to time. Thus with continuous drainage in dog 8, serum calcium rose 9·0 mg. and spinal fluid calcium 3·3 mg. per 100 c.c., and in dog 9 the figures were 4·6 and 3·0 mg. Corresponding figures for the closed system were for dog 11, 5·2 and 1·2, and for dog 12, 4·7 and 0·9 mg. Different animals showed marked variations. The series illustrates the extreme rapidity of adjustment of the blood, since in a dog weighing 20 kg. and having a blood volume of the order of 1800 c.c., dilution with an almost equal volume of solution about four times as strong in calcium content produces only a very transient effect on the blood calcium. The results of these experiments are in good agreement with those of Morgulis & Perley [1930].

The effect of intravenous parathormone injections was relatively slight on the serum calcium, in agreement with previous findings [Thomson & Collip, 1932]. Remembering the normal variations exhibited by the spinal fluid calcium of dog 1, one cannot conclude that there was any definite effect on the spinal fluid calcium of dogs 13 and 14.

The results when parathormone was injected subcutaneously are summarized in Table II. The single experiment with a normal dog in our earlier series [Cameron & Moorhouse, 1925*b*] is listed first in this table. Increases in calcium are expressed in mg. per 100 c.c.

During a period of continuous open drainage the value for a sample of spinal fluid corresponds of course to the (changing) value of the blood

plasma throughout the period of collection, rather than to the value of serum from blood taken at the end of the period.

The figures in Table II illustrate the variable response of serum calcium when similar doses of parathormone are injected into dogs of similar weight, and show equally variable effects for the spinal fluid calcium. With

TABLE II. Summary of results with parathormone

Dog no.	Weight kg.	Parathormone units	Nature of experiment	Duration hours	Maximum increase: Serum Ca (i)	Maximum increase: C.S.F. Ca (ii)	Ratio (ii)/(i) p.c.
(2)	15½	—	Closed system	41	6·1	2·6	43
15	18	130	Continuous open drainage	7½	2·8	0·3	11
16	20	200	" "	25½	4·5	1·2	27
17	18	150	" "	21	5·4	0·7	13
18	23	200	" "	9	2·2	0·7	32
19	27	200	" "	11½	1·5	0·8	53
20	29	200	" "	27	8·2	1·0	12
21	26	200	" "	11	1·7	(1·7)	(100)
22	20	200	Open drainage after 16 hours	20	7·9	2·8	35
23	22	200	" " 16 "	19	6·6	1·1	17
24	19	200	" " 16½ "	23½	3·9	0·8	21
25	21	300	" " 17½ "	21	8·5	0·7	8

the single exception of dog 21 (in which the figure in parentheses depends on a single analysis and cannot be stressed), the rise in the fluid calcium is only a fraction, and usually a small fraction of that in the serum. In only half of these experiments does the actual rise exceed the variations exhibited in the spinal fluid of dog 1, without treatment, during a 5½ hours' period. Nevertheless, careful examination of the figures in Table I permits the conclusion that there is a general upward trend in most of the experiments in Series V and VI. The great variation in response is exemplified by the figures for dogs 16 and 20. In the latter there is a steady slight continuous rise. In the former there is a definite, repeated fluctuation, greater than the normal fluctuation exhibited by dog 1, and perhaps related to the rate of flow of the fluid. Almost every figure in these two experiments is the mean of duplicate analyses which were in good agreement.

Curiously enough the maximum effect on the spinal fluid calcium was not obtained in Series V, with continuous open drainage, but in Series VI, with open drainage only established towards the end of the experiments.

The general results following parathormone injection are also in fairly good agreement with those of Morgulis & Perley [1930].

The results quoted from the literature indicate that parathormone increases both diffusible and non-diffusible fractions of the plasma calcium to approximately the same extent. It must be concluded that the increase in the diffusible fraction is not reflected in the cerebrospinal fluid, even when it is drained off so rapidly that it must be formed from the plasma at a fairly rapid rate. The conditions in this acute hyperparathyroidism thus parallel those in chronic hyperparathyroidism and in other pathological states associated with high serum calcium, and are emphasized by the similar lack of adjustment in experimental and clinical hypoparathyroidism.

The current theory of parathormone action is that it produces a direct denudation of the solid from bone, so that an increased amount of calcium and of phosphate are thrown into the circulation. The phosphate escapes easily (there is no immediate rise in plasma phosphate), but the calcium does not, and should be distributed between the diffusible and non-diffusible fractions in the plasma in the same way as is injected calcium, so that however a hypercalcæmia is produced, its effect on the spinal fluid calcium should be the same. Comparison is difficult on account of the great variation of response in different animals, and the difficulty of maintaining hypercalcæmia by injection of calcium salts. Nevertheless the experiments of Morgulis & Perley [1930] and our own show that with hypercalcæmia produced by either method, change in calcium of the spinal fluid does not correspond with change in diffusible calcium of the plasma.

The present known facts therefore force the conclusion that cerebrospinal fluid calcium only represents the diffusible calcium under normal conditions, and—if indeed this apparent agreement is not fortuitous—we must endorse the view of Morgulis & Perley [1930] that "though the cerebrospinal fluid calcium may be dialysed from the plasma, there must be some additional mechanism involved in governing its rise and fall, which is independent of the physicochemical conditions of the ordinary membrane equilibrium". The "lag" which obviously suggests itself as explanation of differences in acute conditions of hypo- and hypercalcæmia [Greenburg *et al.* 1930] should have been largely excluded by continuous open drainage, and cannot explain those existing in chronic pathological states. Ionic calcium from blood plasma does not appear to diffuse readily through the choroid.

SUMMARY

The ratio of cerebrospinal fluid calcium to serum calcium in dogs with normal blood calcium averaged 50 p.c. for twenty-four animals.

Results of experiments following injections of calcium salts or of parathormone, with or without continuous open drainage from the cisterna magna, lead to the conclusion that the cerebrospinal fluid calcium only represents the diffusible fraction of the plasma calcium when the blood calcium is normal.

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THE EFFECTS OF TETRA-ETHYLAMMONIUM IODIDE ON THE ELECTRICAL RESPONSE AND THE ACCOMMODATION OF NERVE[1]

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Tetra-ethylammonium salts have long been known to differ from most other quaternary ammonium salts in many respects. Two of these are that tetra-ethylammonium salts have only a feeble curariform action and that they produce fibrillary twitching in voluntary muscle [Brunton & Cash, 1884; Boehm, 1910]. Many investigators, some of whom are mentioned below, have established that the muscular twitchings are peripheral in origin. On the one hand Tillie [1890], Jacobj & Hagenberg [1902], Rothberger [1902] and Marshall [1914] have all brought forward evidence that the tetra-ethylammonium ion produces fibrillation by acting upon motor nerve-endings. On the other hand there is evidence, mainly from more recent investigations, of a direct action of the tetra-ethylammonium ion on nerve. Marshall himself stated that the threshold electrical stimulus necessary to excite the motor fibres of the frog's sciatic nerve diminished after the injection of small amounts of tetra-ethylammonium chloride. He also observed that whilst calcium salts would inhibit the twitchings produced in nerve-muscle preparations by tetra-ethylammonium chloride, they had little or no influence upon its curariform action. The experiments were performed both with preparations from frogs which had been injected with calcium chloride previously, and with preparations which were treated with calcium-enriched Ringer's solution containing tetra-ethylammonium

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chloride. Marshall established that tetra-ethylammonium ions do not remove calcium ions from solution through the formation of complexes. Loeb & Ewald [1916] found that immersion of the nerve only of a frog's sciatic-gastrocnemius preparation in Ringer's solution containing 6–42 millimols per litre of tetra-ethylammonium chloride caused twitching of the muscle, and that the onset of the twitching could be delayed or even prevented by the addition of calcium salts to the solution with which the nerve was treated. Unlike Marshall, they found no lowering of the threshold to induction shocks after treatment with tetra-ethylammonium chloride; in fact, they also found no change in the threshold of nerves that had been treated with tetra-ethylammonium chloride together with sufficient calcium chloride to prevent the development of twitchings of the muscle. They found that in nerves that had been treated with tetra-ethylammonium chloride-Ringer's solution in which part of the sodium chloride had been replaced by glucose the threshold was raised considerably; this procedure also inhibited the development of twitching in the muscle.

Cowan & Ing [1933, 1935] observed that in frog's isolated sciatic nerve, stimulated repetitively for short periods by maximal condenser shocks, the action current, measured ballistically as the area under a galvanometer deflexion and time curve, was increased from 50 to 100 p.c. after soaking in Ringer's solution containing 10 millimols per litre of tetra-ethylammonium iodide.

In the experiments described in this communication we examined, with an amplifier and oscillograph, the electrical response of nerve which had been treated with tetra-ethylammonium iodide. As we had anticipated, the response proved to be repetitive. This result led us to examine the nature of the changes of excitability responsible for the repetitive response. In this communication we have not attempted to deal with the action of tetra-ethylammonium iodide on the nerve-muscle preparation. This relatively more complex system than nerve will be discussed by one of us (S. L. C.) in a subsequent paper.

Materials

The frogs were English *Rana temporaria*, and except where otherwise stated, they were caught between mid-March and the end of May, and used within a fortnight of capture. Ringer's solution of the following composition was made up with "AnalaR" chemicals and water distilled in a porcelain still: NaCl 0·65, KCl 0·10, $CaCl_2$ 0·20, water to 100 c.c., and

contained 10 mg. per 100 c.c. of phosphorus in the form of phosphate buffer to maintain the *p*H at 7·2. The tetra-ethylammonium and tetramethylammonium iodides used were purified by recrystallization from absolute alcohol. The Ringer's solutions to which either of these substances had been added were diluted with distilled water to restore their original tonicity.

The electrical responses of nerve

Experimental

The sciatic nerve trunk was mounted in moist air (17–20° C.) in a closed ebonite chamber, and lay on four electrodes consisting of silver wires coated with silver chloride over which had been wound, just before the beginning of the experiment, thick thread soaked in Ringer's solution.

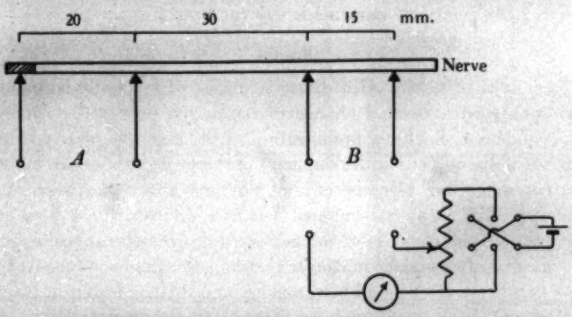

Fig. 1. The arrangement of the electrodes on the nerve trunk. *A*, leading-off electrodes to amplifier. *B*, electrodes for stimulus or polarizing current.

The purpose of the thread was to prevent the nerve making contact with the silver chloride, and the consequent disturbing action described by Cowan & Ing [1935]. One pair of electrodes was used for leading off to the input of an amplifier, a monophasic response being secured by first crushing the peripheral end of the nerve and then applying a drop of isotonic potassium chloride solution; the other pair of electrodes was used to apply stimuli or small polarizing currents to the nerve (Fig. 1).

The amplifier was resistance capacity coupled and had a time-constant of approximately 1 sec. The output was fed to a Matthews's moving-iron oscillograph, through the special output stage devised by Matthews [1928]. The action potentials were examined on the screen of a "standing

wave" camera similar to that described by Matthews [1929], but modified in one respect. Instead of the single contact breaker and cam on the driving shaft to give stimuli synchronized with the mirror, there were two cams operating two contact makers—one fixed and one adjustable. These enabled condenser charge and discharge shocks to be applied, and by altering the angular distance between them and watching the action potentials on the viewing screen it was easy to determine the refractory period of the nerve. The action potentials were recorded, when desired, on ciné bromide paper.

The stimuli usually applied to the nerve were condenser shocks ($RC = 250$ μsec.) and the voltage to which the condenser was charged was adjusted with a tapped battery in series with a potentiometer connected across a single cell. The shocks were just maximal except in the determinations of the absolute refractory period. The method of obtaining the polarizing currents is evident from Fig. 1.

Results

Treatment of nerves with dilute solutions of tetra-ethylammonium iodide produced a twofold change in the action potential response to single condenser shocks: a prolongation of the negative after potential; a repetitive discharge. The development of these effects is shown in Fig. 2. *A* is the monophasic response of a nerve trunk which had been soaked in aerated and phosphate-buffered Ringer's solution for $\frac{1}{2}$ hour after dissection; *B*, the response of the nerve trunk from the opposite side of the same frog after soaking in Ringer's solution for 2 hours; *C*, the response of the first nerve after it had been soaked for a further $\frac{1}{2}$ hour in Ringer's solution containing 10 millimols per litre of tetra-ethylammonium iodide; *D*, a response recorded under the same conditions as *C*, but with greater amplification; *E*, the response in the same nerve after it had been soaked for another hour in the 10 millimolar tetra-ethylammonium iodide solution, then re-mounted and a fresh injury made; *F*, taken at a slower speed, the response about 5 min. after painting the nerve with a 42 millimolar solution of tetra-ethylammonium iodide in Ringer's solution. From the records it can also be seen that the repetitive discharge began synchronously and became progressively more asynchronous.

In nerves which were examined after immersion for $\frac{1}{2}$ hour in 10 millimolar tetra-ethylammonium iodide solution no change was detected in the absolute or relative refractory periods, or in the threshold to the short stimuli employed. Further, in the records, the distance between the stimulus escape and the beginning of the action potential, that is the

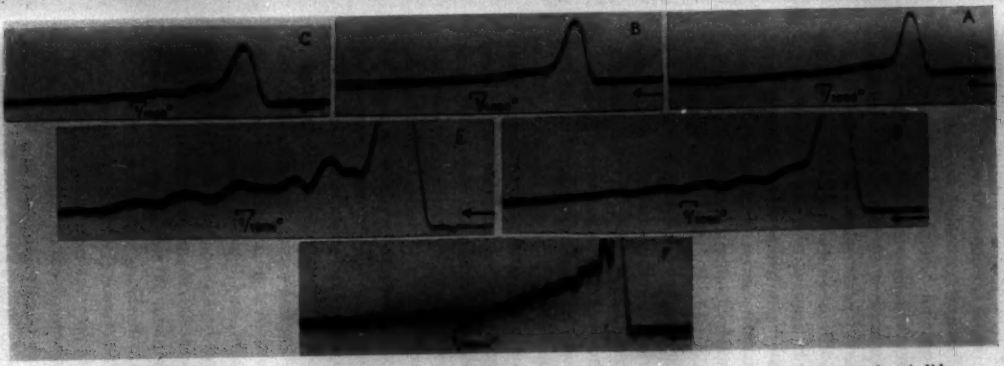

Fig. 2. Monophasic records of action potentials in frog nerve before and after treatment with tetra-ethylammonium iodide Ringer solution. Explanation in the text.

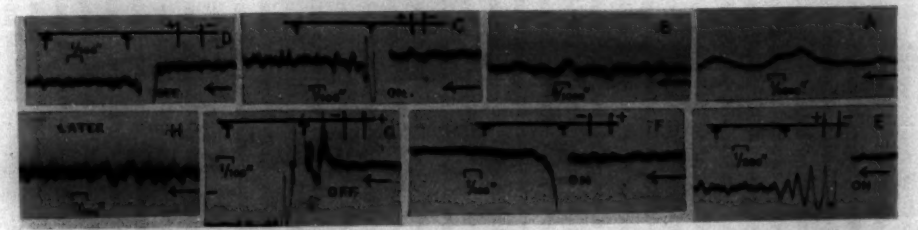

Fig. 3. Monophasic records of "spontaneous" activity in nerve after treatment with tetra-ethylammonium iodide solutions, and the influence of polarizing currents on the activity. Details in the text.

latent period of the nerve plus the conduction time, remained unchanged after tetra-ethylammonium iodide. From the constancy of the threshold to short shocks we infer that the "utilization time" was also constant and that it is probably justifiable to conclude that the conduction velocity was not affected by the dilute tetra-ethylammonium iodide solution.

Immersion of a nerve for 5–10 min. in a 42 millimolar tetra-ethylammonium iodide solution produced "spontaneous" asynchronous activity; nerves immersed in more dilute solutions (15–30 millimolar) developed such activity after longer times (40–20 min.). Later in the year, June–July, nerves which had been immersed in 10 millimolar solution often developed "spontaneous" activity provided that they had not been subjected to preliminary soaking in Ringer's solution (0·02 p.c. $CaCl_2$) for any considerable time. Fig. 3*A*, taken at the same speed as Fig. 2*A–E* and with amplification three times greater than in *E*, is a record of the activity in a nerve that had been treated with the 42 millimolar solution of tetra-ethylammonium iodide. Fig. 3*B*, taken at the same speed as Fig. 2*F*, shows the form of the discharge more clearly. Records taken with a control nerve which had been immersed in Ringer's solution gave a perfectly steady base line.

Examination of the effects of constant currents on the discharge in nerves which were in a state of moderate "spontaneous" activity showed that the normal electrotonic changes of excitability at the anode and at the cathode, i.e. Pflüger's changes of excitability, had become reversed. For the records in Fig. 3*C–G* the current was one-quarter of the rheobase which was found for the normal nerve before treatment with tetra-ethylammonium iodide solution. Even this small current, approximately 0·25 μA., seems to have been a little above the threshold in these experiments. [Measurements, described later, with calomel half-cells as non-polarizable electrodes confirmed the lowering of the rheobase.] With the anode nearer to the leading-off electrodes the making of the current excited a small group of fibres. Fig. 3*C*, and Fig. 3*E* taken at a higher speed than 3*C*, show that the discharge began synchronously and became progressively more asynchronous in the 20–30 msec. immediately after the closure; from then onwards for about 30 sec. the "spontaneous" activity was considerably augmented. Breaking the current during the period of augmented "spontaneous" activity excited a few fibres and then the discharge rapidly sank to its previous resting value (Fig. 3*D*). With the polarity reversed a few fibres were excited on making the current, then the "spontaneous" activity was rapidly inhibited and remained so during further passage of the current (Fig. 3*F*). At the break again,

a few fibres were excited and there was an outburst of "spontaneous" activity which declined slowly (20 sec.) to its resting level (Fig. 3*G–H*).

The effects of polarizing currents could be observed equally well whether or not the part of the nerve between the leading-off electrodes had undergone treatment. This suggests that the reason why augmentation or inhibition effects were observable was that the impulses affecting the amplifier and oscillograph were chiefly those coming from the part of the nerve outside the leading-off electrodes and that impulses arising in that part of the nerve between the leading-off electrodes were distributed symmetrically giving only a very small net action potential.

Similar experiments to those already described, but with nerves that had been immersed for up to 2 hours in 10 millimolar tetramethylammonium iodide solution, showed no change in the action potential and no sign of "spontaneous" activity.

Measurements of accommodation in nerve

Loeb [1901] found that treatment of the nerves only of frog's nerve-muscle preparations with solutions of sodium salts whose anions remove calcium ions from solution, e.g. fluoride, carbonate, phosphate (Na_2HPO_4), oxalate, citrate or tartrate, set up twitchings in the muscles. He found too that the twitchings could be stopped by the addition of a sufficient quantity of calcium chloride. Further experiments led Loeb to the conclusion that the salts in question did not stimulate the nerve, but rather rendered it sensitive either to a contact or a mechanical stimulus—both, as Loeb employed them, stimuli of long duration. Mines [1908], who examined the "spontaneous" twitchings and changes of excitability of the frog's sartorius muscle when placed in calcium-free Ringer's solution, was able to put Loeb's conclusions into more quantitative terms. Like Loeb, Mines found that the threshold to break induction shocks was unchanged, but Mines also found that the threshold to constant currents of long ($\frac{1}{2}$ sec.) duration, in fact what we now call the "rheobase", was reduced to about one-third of its normal value.

Lucas [1907] established that each of the tissues, muscle and nerve, requires a characteristic "minimal current gradient" for its excitation, and later showed that in his own experiments and in those of Mines [1908, and reported in Lucas, 1908], the increase of rheobase produced by treatment with calcium-rich solutions was accompanied by a corresponding increase of the "minimal current gradient". Subsequently, Lucas [1910] drew attention to the association between "spontaneous" activity and the diminutions of "minimal current gradient" and of

rheobase. Kahn [1911], in experiments on the excitation of the intramuscular nerve fibres of the frog's isolated sartorius by currents increasing exponentially to steady values, also found that rates of rise, slower than those effective with preparations which had been soaked in normal or calcium-rich Ringer's solution, sufficed for preparations which had been soaked in calcium-deficient solutions.

Hill [1936] has pointed out that excitation by electric currents involves two independent time factors. For theoretical treatment he has assumed that the time factors are the time-constants of two exponential processes—a fast one and a slower one—whereby an excitable tissue reverts to its resting state upon the instantaneous removal of a subthreshold current. According to his theory, excitation by current of short duration involves only the shorter factor k, the time-constant of excitation, whereas excitation by slowly rising currents or currents near to the rheobase involves in addition λ, the time-constant of "accommodation". Also according to the theory, repetitive response is a consequence of slow "accommodation", i.e. long λ [see Katz, 1936].

In the part of Hill's theory which deals with excitation by currents increasing exponentially to constant values it is predicted that if the exciting current, instead of reaching the threshold value instantly, increases exponentially, then the threshold exponential current (I) divided by the rheobase (I_0) should bear a nearly linear relation to the time-constant of current rise (α), so long as α and λ are both large compared with K. The slope of the line should be $1/\lambda$ where λ is the time-constant of "accommodation" defined above. Hill has pointed out also that $1/\lambda$ should be equal to Lucas's "minimal current gradient". On testing experimentally the relation between I/I_0 and α Solandt [1936] found that it was linear, for values of α that were sufficiently large not to infringe the condition just stated. Changing of the calcium-ion concentration of the fluid bathing frog nerve caused the slope of the line to change through a wide range of values, but produced no departure from linearity. He concluded that the action of calcium ions in diminishing λ is almost a specific one and that k is changed comparatively little. He demonstrated also that removal of calcium ions from nerve by immersion in sodium citrate or oxalate solution caused λ to become very large and finally infinite when "spontaneous" activity began.

We anticipated that, like the agencies mentioned above, tetra-ethyl-ammonium ions would reduce the rheobase and would cause nerve to become more easily excited by slowly rising currents. It remained to determine whether the relation between I/I_0 and α was a linear one, and

if it was linear, whether the changes of λ so measured, together with changes of rheobase, would account for the repetitive responses and "spontaneous" activity produced by tetra-ethylammonium iodide.

Experimental

Our method of obtaining currents which rose exponentially, to constant values, with known time-constants of rise (α) was essentially the same as that employed by Solandt [1936] in his experiments in frog nerve. Since measurement of the small currents necessary for excitation would have prevented readings from being made in rapid succession we also adopted Solandt's plan of measuring the thresholds in volts at the potential source. The values of E/E_0 actually obtained from our experiments were the same as those that would have been measured by I/I_0, because the resistance of the circuit used was constant during any one set of observations and the current was proportional to the applied voltage.

The experiments were performed at room temperature (17–19° C.) with gastrocnemius-sciatic preparations which had been soaked for 1½–2 hours in Ringer's solution. A paraffin wax chamber with a closely fitting glass cover was used; moist air or oxygen could be passed through the chamber. The preparation lay horizontally, the muscle on the floor of the chamber and the nerve on two waxen shelves on which had been placed two filter paper strips (each 4 mm. wide and with 14½ mm. between their nearer edges) connected to large calomel electrodes used to apply the exponentially increasing currents. The presence of the minimal twitch, which was used as an indication of excitation in the motor nerve fibres, was detected by the movement of a fragment of silvered microscope cover-slip resting on the belly of the muscle. A beam of light was arranged so that it fell on the mirror and gave a spot on a ground glass scale.

Measurements were begun by determining the threshold voltage for a current of instantaneous rise (the rheobase), then the thresholds for currents of longer and longer time-constant of rise were determined. A return series, ending with the rheobase, was also performed, thus giving two thresholds which could be averaged for each time-constant of current rise. Throughout, the trials were made with inadequate stimuli which were increased until the threshold was reached. Finally, the effective resistance shunting the condenser was measured by substituting a low-resistance galvanometer in series with a small known E.M.F. in place of the condenser. When the first part of the experiment had been completed

the preparation was taken out, a small weight attached to the central end of the nerve, which was then put into the tetra-ethylammonium-iodide-containing solution, the effect of which it was intended to examine. A closed vessel was used so that the muscle was suspended in moist air and care was taken to prevent contact with the walls since any partial paralysis of the preparation by the quaternary salt would have vitiated the experiment. After a definite time had elapsed the muscle was rinsed with Ringer's fluid and the surplus drops wiped off. The preparation was re-mounted and a second series of measurements made. In some cases after the nerve had been soaked in a second solution a third series of measurements was made. The experiment was completed by the testing of a control preparation which had been taken from the opposite side of the same frog and which had been soaking in Ringer's solution.

Results

The relation between E/E_0 and α was substantially a linear one, even for nerves which had been treated with concentrations of tetra-ethyl-ammonium iodide sufficient to cause "spontaneous" activity to begin soon after the measurements had been made (e.g. Fig. 4 *B* and *C*, Fig. 5 *B* and *C*). Also, for all but large values of the slope of the line in question, i.e. with nerve treated with tetra-ethylammonium iodide, when short durations of α were employed there was curvature corresponding to a more rapid diminution of E/E_0 than of α; for large values of the slope, i.e. for Ringer-soaked but otherwise untreated nerve, the relation remained linear or nearly linear when short durations of α were employed. The first two results are of types which accord with Hill's theory and we have therefore concluded that, for nerve treated with tetra-ethyl-ammonium iodide, it is permissible to refer to the slope of the linear part of the curve relating E/E_0 and α as λ, the "time-constant of accommodation" of the theory. The third result no more than confirms Solandt's finding that when λ is small, then with small values of α, the curvature predicted by the theory is absent. Hill has pointed out (pp. 322 and 337) that the values of λ determined by this method are rather too large, and require correction, because the "observed" rheobase is slightly higher than the "true" rheobase. However, we have omitted the corrections throughout this paper, because they would have been small in comparison with the changes of λ studied [for calculation see Solandt, 1936], and, in the case of Ringer-soaked nerve, not of a kind to remedy the deviation from the predicted relation.

In experiments performed mainly during the first 2 weeks in May, values of λ between 10 and 18 msec. were found for nerves that had been soaked in Ringer's fluid for 1½–2 hours (e.g. Fig. 4 *A*, Fig. 5 *A*), and between 13 and 20 msec. for nerves that had been soaked for 3–5 hours (e.g. Fig. 4 *D*, Fig. 5 *D*). Before use the frogs were kept at the laboratory

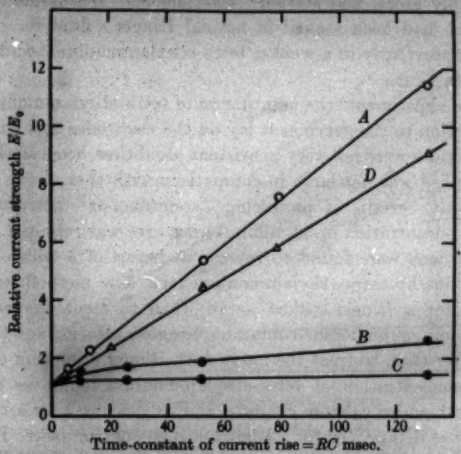

Fig. 4. "Accommodation" curves for frog nerve. *A*, nerve had been soaked in Ringer's fluid for 1½ hours. $\lambda = 12{\cdot}7$ msec. *B*, after the same nerve had been immersed for ½ hour in Ringer's fluid containing 10 millimols per litre of tetra-ethylammonium iodide, and then remounted. $\lambda = 122$ msec. The nerve was then immersed for 15 min. in 30 millimolar tetra-ethylammonium iodide Ringer's solution, re-mounted, and left undisturbed for 10 min. before the determinations. *C*, these were made as quickly as possible as the nerve was not in a steady state. $\lambda = 588$ msec. Less than ½ hour later λ had become immeasurably long and there were occasional "spontaneous" discharges. *D*, control nerve that had been soaking in aerated Ringer's fluid for 3¼ hours. $\lambda = 15{\cdot}5$ msec.

temperature (16–20·5° C.). No measurements were made on nerves immediately after dissection. Our values of λ are slightly smaller than Solandt's for Ringer-soaked nerve—probably because our experiments were made not long after the breeding season, whilst his experiments extended over a longer period.

If present in sufficient concentration, tetra-ethylammonium iodide reduced the power of "accommodation" of nerve to vanishing point. Fig. 4 gives the results of an experiment.

For nerves that had been soaked in Ringer's solution for 1½–2 hours and then for ½–1 hour in Ringer's solution containing 10 millimols per litre of tetra-ethylammonium iodide, λ lay between 100 and about 600 msec. Immersion of Ringer-soaked nerves in 15 millimolar tetra-ethylammonium iodide Ringer's solution for about ½ hour made λ immeasurably great, and started "spontaneous" discharges, except in a few which had been soaked in normal Ringer's fluid for more than 3 hours, or previously in a weaker tetra-ethylammonium iodide solution for about an hour.

In a few experiments the painting on of tetra-ethylammonium iodide Ringer solution to the nerve as it lay on the electrodes was tried. However, the results were not very consistent, doubtless because the volume of fluid applied was not large in comparison with that of the nerve. To be reasonably certain of producing "spontaneous" activity by this method a concentration of 30 millimols per litre was required.

Calcium ions were found to reverse increases of λ which had been brought about by tetra-ethylammonium ions. The method was first to measure λ for a Ringer-soaked nerve; then to treat the nerve with tetra-ethylammonium iodide-containing Ringer's solution and to measure λ again; after that, to treat the nerve with Ringer's solution containing the same concentration of tetra-ethylammonium iodide as previously together with added calcium chloride, and to measure λ for a third time. Finally, λ was determined for a Ringer-soaked control nerve. The results of one such experiment are summarized in Fig. 5. Concentrations of calcium chloride 2–3 times that of normal Ringer's fluid (0·02 p.c.) were sufficient to reverse the changes produced by 10 millimolar tetra-ethylammonium iodide solution.

An adequate concentration of calcium chloride applied simultaneously with tetra-ethylammonium iodide could prevent the changes of λ that would have been produced by the quaternary salt alone. In two experiments Ringer-soaked nerves were immersed for 2½ hours in a solution containing 10 millimols per litre of tetra-ethylammonium iodide and 0·08 p.c. of calcium chloride (7·2 millimols per litre): in both of them λ, initially about 14 msec., was changed by less than 15 p.c.

In the various kinds of experiment described above a diminution of "accommodation" was accompanied by a diminution of rheobase. When λ was increased to 300–500 msec. the rheobase was lowered to about one-half of the value found before treatment with tetra-ethylammonium iodide solution; when λ had become immeasurably long, in the intervals between "spontaneous" discharges, the rheobase had a value 1/4 to 1/8

of the normal one (1·0–1·8 μA.). The lowerings of rheobase were reversed or prevented by calcium chloride in concentrations sufficient to reverse or prevent the changes of λ produced by tetra-ethylammonium iodide.

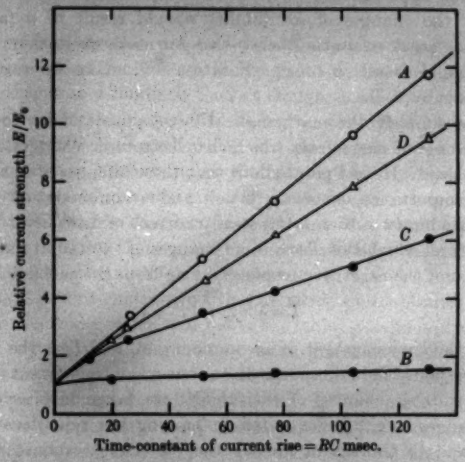

Fig. 5. The reversal of "accommodation" changes by calcium chloride. *A*, nerve which had been soaked in Ringer's fluid for 2 hours. λ = 12·0 msec. *B*, after the same nerve had been treated for an hour with Ringer's solution containing 10 millimols per litre of tetra-ethylammonium iodide. λ = 323 msec. The nerve was then immersed for an hour in Ringer's solution containing 10 millimols per litre of tetra-ethylammonium iodide and 0·04 p.c. calcium chloride. *C*, λ determined subsequently = 30·7 msec. *D*, control nerve soaked in Ringer's solution for 5 hours. λ = 15·9 msec.

Apparently with tetra-ethylammonium-iodide-treated nerve, as Katz [1936] found with sodium-citrate-treated nerve, the onset of "spontaneous" activity is the limiting result of reduction of the rheobase and of extremely slow "accommodation".

The effects of constant currents

Ordinarily in nerve when a constant electric current just sufficiently strong to excite at make is broken, no further excitation results. If, however, the strength of the current is increased to several times that required for excitation at make, then excitations are obtained both at make and at break. Apparently the threshold for break excitation is

higher than that for make excitation. The phenomena have been explained as a consequence of the rise of excitability which occurs at the cathode and of the fall of excitability which occurs at the anode of a polarizing current when applied before a test stimulus. According to this view, reversal of the changes of excitability should result in a fall of the threshold for break excitation below that for make excitation; abolition of them should result in break excitation and make excitation being obtained equally well.

Hill, to simplify the mathematical development of his theory, has treated the case of nerve from which the electrotonic changes of excitability are absent. He has pointed out that these changes are probably of secondary importance, since excitation and accommodation can take place independently of them. If a steady current of intensity I is applied to a nerve from which the electrotonic changes of excitability are absent, the duration of the repetitive response set up from the cathode should be

$$T = \lambda \log_e I/I_0,$$

where λ is the time-constant of accommodation, and I_0 is the rheobase. The same response should occur at the anode when the current is broken. If the electrotonic changes of excitability are taken into account, the normal changes might be expected to prolong the repetitive response at make, and to shorten or abolish the repetitive response at break; the reversed changes might be expected to have the opposite effects. On comparing the "observed" and "calculated" durations of repetitive response to constant currents Katz [1936] found an approximate agreement between them, but that the "observed" durations might be modified by the electrotonic condition of the nerve, or by changes of refractory period; also, that reversal of the normal electrotonic changes of excitability by cold resulted in the threshold for break excitation falling below that for make excitation.

Since our experiments on the reversal of the normal electrotonic changes of excitability at the anode and at the cathode referred to sciatic nerve trunks in a state of "spontaneous" activity, and our experiments on "accommodation" and rheobase to motor fibres only, we have made further experiments on the thresholds of motor fibres for make excitation and for break excitation, and on the influence of current intensity and direction on the duration of repetitive response to constant current. Nerve-muscle preparations were used, and, as at the time of these experiments the amplifier and oscillograph were not available, the mechanical response of the muscle was taken as a measure of the

response of the motor nerve fibres. Our observations, therefore, on the duration of repetitive response elicited from nerve are only semi-quantitative. The import of this limitation will be discussed later.

Experimental

Sciatic-gastrocnemius preparations, from winter frogs kept at 14–18° C., were soaked for 2 hours in Ringer's solution and then mounted in moist oxygen in a modified Lucas's trough closed by a cover through which the lever crank passed. The femur was held in a clamp, and the tendon was tied to the crank which was also connected to an isometric lever which, when writing on smoked paper, had a period of 1/30 sec. The central part of the nerve was supported on wicks leading to two calomel half-cells: the diameter of each wick was about 1·2 mm., and the distance between them was 20 mm.; the distance between the wick more distal from the muscle and the cut (central) end of the nerve was 6–10 mm. Constant currents in either direction were obtained as follows. One of the electrodes was connected to the moving contact of a graduated potential divider through a 200,000 ohm "metallized" resistance, the other electrode was connected directly to the fixed contact of the potential divider, thus completing the circuit. The ends of the potential divider were connected to a 4 V. battery through a reversing key. The resistance of the nerve and electrodes was measured and found to be 90–100,000 ohms, from this and the other constant of the circuit the current through the nerve was calculated. After the preparation had been mounted the experimental procedure was to determine the rheobase of the nerve and then to try currents of several times the rheobase until break excitation was observed. To avoid changes in the condition of the nerve due to the constant currents they were passed in descending and ascending directions alternately. When desired, records of the muscle response were made. After the preliminary determinations were finished the nerve was lifted off from the electrodes, without disturbing the muscle, and the central half immersed in tetra-ethylammonium iodide Ringer solution. The wicks leading to the electrodes were also immersed in the solution with which the nerve was treated. At the end of a definite time the nerve was re-mounted and fresh tests made.

Results

In the following paragraphs the expression "threshold for make excitation" or "make threshold" means the intensity of descending current necessary just to excite the motor nerve fibres at make, i.e. with

the cathode as the electrode nearer to the muscle; "break threshold" refers to current ascending nerve.

Fig. 6. The responses of a nerve-muscle preparation to constant currents applied to the nerve, before and after it had been treated with tetra-ethylammonium iodide. The time markings are in seconds; depression of the signal line indicates make of the current and rise indicates break of the current. The preparation was mounted after 2 hours soaking in Ringer's solution, and the threshold current for excitation of the motor nerve fibres at make was determined periodically. In ½ hour it fell from 1·3 to 1·1 μA. and remained constant for the following ½ hour. The break threshold was about (7 × 1·1) μA. *A*, response to descending current five times make threshold (1·1 μA.), recorded at 65 min. (this and the other times below are reckoned from the mounting of the preparation). *B*, response to ascending current five times make threshold, recorded at 66 min. The nerve was immersed in 30 millimolar solution from 70 until 93 min. and then re-mounted. Irregular outbursts of "spontaneous" activity were beginning to appear, and determinations of threshold, which could only be made in the quiescent intervals, became inexact. The average make threshold was about 0·3 μA.—a quarter of its former value; the average break threshold was about 0·25 μA.—one-thirtieth of its former value. *C*, response to descending current three times make threshold, recorded at 100 min. *D*, response to ascending current three times make threshold. At 130 min. the make and break thresholds were approximately equal (0·3 μA.). *E*, response to descending current five times make threshold (0·3 μA.), recorded at 133 min. *F*, response to the same current ascending.

Ringer-soaked nerves.

The condition of the nerves used in our experiments was such that a current intensity of 5–9 times the threshold for make excitation was required to produce excitation at break. The muscle responses elicited

at the make of currents of intensity up to seven times the (make) threshold were ones corresponding in size to a few (usually 1–3) maximal volleys in the motor fibres of the nerve. The size was independent of the direction of current flow (cf. Fig. 6*A* and *B*, Fig. 7*A* and *B*).

The influence of tetra-ethylammonium iodide on threshold.

The threshold for make excitation was reduced considerably, and the threshold for break excitation to a greater extent. In the case of nerve that had been treated with 30 millimolar solution determinations of threshold were made in the intervals between "spontaneous" discharges. Soon after treatment the break threshold fell below the make threshold; later, the break threshold rose again slightly. In nerves treated with smaller concentrations of tetra-ethylammonium iodide (10–15 millimols per litre) the break threshold never fell below the make threshold, nor was there a later rise of the break threshold relative to the make threshold.

The effects of tetra-ethylammonium iodide upon responses to descending currents 2–7 times threshold.

Following the make of a current applied to a nerve soon after it had been brought to a state just short of "spontaneous" activity by treatment with 15 millimolar solution the response elicited from the muscle was a tetanic one which continued until the break and then stopped (Fig. 7*C*). With nerves which had been treated with 30 millimolar solution, in the intervals between "spontaneous" discharges, the response was also a tetanic one, but there were superimposed small irregularities and it stopped rather suddenly after several seconds although the current was continued for longer (e.g. Fig. 6*C*). In a few instances further activity occurred in the interval between the end of the main response and the break of the current. Presumably this activity was "spontaneous" as it did not appear on applying the test current again some minutes later. The duration of response at make was greater with greater current intensity. After a time the response of preparations whose nerves had been treated with either of the concentrations of tetra-ethylammonium iodide grew progressively more irregular (e.g. Fig. 6*F*, Fig. 7*F*) and, to currents of constant strength relative to the (make) threshold, shorter. Eventually, the response began like a tetanus and quickly resolved into small twitchings which became infrequent and stopped after one or a few seconds although the current was continued for longer (e.g. Fig. 7*G*). The transition from the earlier types of response to the later type was

generally more rapid in "spontaneously" active nerves or nerves which had been subjected to a number of test stimulations (1–2 hours) than in nerves which suffered little activity (2–3 hours). The duration of the later type of response was also dependent on the intensity of the applied

Fig. 7. The responses of a nerve-muscle preparation to constant currents applied to the nerve before and after it had been treated with tetra-ethylammonium iodide. The time markings are in seconds; depression of the signal line indicates closure of the current, rise indicates break of the current. The preparation was mounted after 1 hour 40 min. soaking in Ringer's solution. The make threshold remained steady at 1·4 μA. for an hour. The break threshold was about (9 × 1·4) μA. *A*, response to descending current three times make threshold, recorded 70 min. after mounting. *B*, response to ascending current, three times make threshold. *C*, response to descending current, five times make threshold (5 × 1·0) μA., recorded after the nerve had been immersed for 20 min. in 15 millimolar tetra-ethylammonium iodide Ringer's solution, and left mounted for 7 min. *D*, response to ascending current, five times make threshold. *E*, response to descending current, five times make threshold (5 × 0·8) μA., 20 min. after *C*. *F*, response to ascending current five times make threshold. During the next 90 min. the nerve was painted three times with fresh tetra-ethylammonium iodide solution. *G*, response to descending current, six times make threshold (6 × 0·6) μA., recorded 104 min. after *E*. *H*, response to ascending current, six times make threshold. At this time the break threshold was about (5·5 × 0·6) μA.

current. The greater the intensity: the longer did the initial tetanic part last before breaking up; the longer was the duration of the intermittent twitching; the shorter were the intervals between the earlier of the small twitches.

Excitation following break appeared only with intensities 5–7 times (make) threshold, and was very small (e.g. Fig. 6*E*). This difficulty of obtaining a response at break of descending current occurred with nerves

in which the break threshold had been reduced below, or made equal to, the make threshold, after treatment with 30 millimolar tetra-ethyl-ammonium iodide solution; with nerves in which the break threshold remained above the make threshold, after treatment with 15 millimolar solution (Fig. 7 *C* and *G*).

The effects of tetra-ethylammonium iodide on responses to ascending currents 2–7 times threshold.

Upon testing nerves at various times after treatment with 30 millimolar solution the muscle response following the make of an ascending current resembled qualitatively that following the make of a descending current in that it was a tetanic one which went on for a few seconds and then stopped quite suddenly although the current was maintained for longer, but differed quantitatively in that the duration and tension were both smaller than when the current was descending (cf. Fig. 6 *C* and *D*, Fig. 6 *E* and *F*). The response to ascending current lasted longer the greater the intensity of the current. The difference between the responses at make of ascending and of descending current was more marked with preparations whose nerves had been treated with 15 millimolar solution: the response with ascending current was of a size that would have been produced by a few maximal volleys in the untreated nerve (cf. Fig. 7 *C* and *D*, *E* and *F*, *G* and *H*).

Following the break of ascending current, preparations whose nerves had been treated with 30 millimolar solution gave a tetanic response which came to a well-marked stop, and it was seldom that any further excitation occurred for several seconds afterwards (e.g. Fig. 6 *F*). The duration of the break response was greater the greater had been the intensity of the applied current, but was always shorter than that of the make response preceding. For nerves which had been treated with 15 millimolar solution the ratio of break threshold to make threshold, although diminished, had values from 3 to 6, consequently break excitation was absent with the lower current intensities, and small with the higher intensities which we used (Fig. 7 *D*, *F* and *H*).

In the course of each experiment, as happened with the responses at make of descending current of constant intensity relative to the (make) threshold, the responses at make and at break of ascending current grew shorter and more irregular.

(i)

The possibility that failure of the transmission of excitation from nerve to muscle might have modified the tetanic responses described under the two preceding subheadings must be considered with respect to three phenomena.

(1) The relatively sudden ending of certain responses (e.g. Fig. 6*C* and *D*). The observed increase of duration of these responses with increase of applied current (which would have meant a greater initial frequency of nerve volleys owing to excitation earlier in the relative refractory period) is incompatible with junctional failure.

(2) The initial decline of tension in responses elicited by currents of high intensity (e.g. Fig. 6*C* and *D*, Fig. 7*C*). Probably this decline was due to partial failure of junctional transmission because with increased current it occurred earlier and was more marked.

[The simultaneous influence of the phenomena of 1 and 2 upon responses can perhaps be understood better by taking an example. In Fig. 6*D*, in the response following make of an ascending current three times threshold, there is a sudden fall of tension in the first second. A record of a response to a current five times threshold was made a few minutes after that shown in Fig. 6*D*. With the greater current the response lasted (the method of measurement is given below) 8·1 sec. against the 5·3 sec. of that in Fig. 6*D*, but the initial fall began earlier and amounted to about 30 p.c. of the maximum tension developed.]

(3) The intermittency and eventual failure in the later type of response. The effects of increase of current intensity on these indicate that they originated in the nerve and not in the neuromuscular junction. In the later responses apparently the initial tetanic part corresponds to synchronous excitation of the motor units of the preparation, and the smaller twitchings to asynchronous and infrequent excitation of single motor units or groups of motor units. Katz found that the response of sodium-citrate-treated nerve, which had been "spontaneously" active for some time, to constant current, became intermittent and failed rapidly. He has suggested that these events are due to lengthening of the refractory period, mainly at the point of stimulation [Bugnard & Hill, 1935].

(ii)

In two of our experiments with preparations whose nerves had been treated with 30 millimolar tetra-ethylammonium iodide solution attention was given to the effect of current intensity on the duration of repetitive response. Tests were begun soon after "spontaneous" activity appeared and continued as nearly in accordance with the following scheme as was consistent with avoiding the "spontaneous" outbursts. The response at make of a descending current was first recorded and then a few seconds later the response (if any) at break. About 3 min. later the responses at make and at break were recorded with the same current ascending. After another 5 min. the responses to a current of higher intensity were recorded.

Before stating our results it must be pointed out that the duration of excitation of the motor nerve fibres could most nearly have been measured as the interval between the beginning of the first and of the last action potential of the muscle; that we were concerned more with comparing the durations of excitation with currents of different strengths than with the absolute durations; that granting the interest of our experiments to lie mainly in the comparison, our use of the mechanical response of the muscle involves approximations which have to be described and examined. Although the ending of responses such as those of Fig. 6*C* and *D* was well marked, the beginning of the final fall of tension, which might have served as a rough indication of the end of excitation, was not sufficiently marked to be used for our measurements. Therefore, to obtain a well-defined end-point, even at the cost of including part of the relaxation, we have adopted the somewhat arbitary procedure of producing the line representing the final fall of tension until it cut the horizontal line of zero tension.

The two experiments yielded a practically linear relation between the logarithm of the current intensity relative to the threshold (I/I_0) and the duration of response (measured to our arbitrary end-point): at make of descending current; at make of ascending current; at break of ascending current. The results of one of the experiments (the one from which the records shown in Fig. 6 have been taken) are summarized in Fig. 8. It is to be noted that we have drawn the three straight lines in the figure as passing through the origin, although other straight lines lying equally well among the small number of experimental points and cutting off small positive intercepts on the ordinate axis might perhaps have

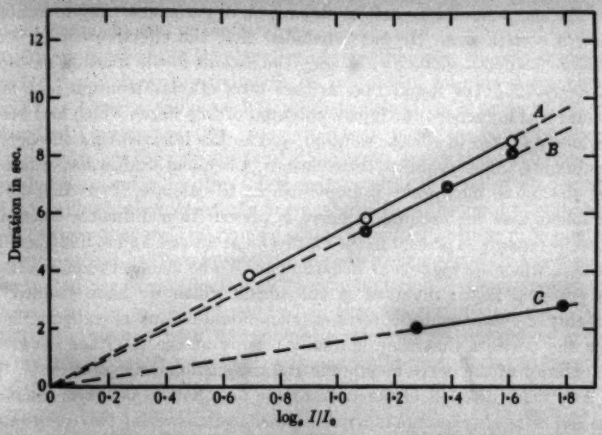

Fig. 8. The relation between the duration of repetitive response given by a nerve-muscle preparation, and the strength of current applied to the nerve, which had been treated with 30 millimolar tetra-ethylammonium iodide solution a short time before the tests. *A*, at make of descending current (strength relative to the make threshold). From the slope of the line, the time-constant of cessation of response at the cathode is 5·3 sec. *B*, at make of ascending current (strength relative to make threshold). *C*, at break of ascending current (strength relative to break threshold), whence the time-constant of cessation of response at the anode is 1·6 sec.

been drawn. It is impossible to say whether we have done rightly. An intercept would have been expected, on account of the part of the muscle relaxation process included in each of our measurements of duration, had not two other factors been operating: the well-known slowing of relaxation after activity [see Hill, 1931; Feng, 1931; Parkinson, 1933], which would have increased the slope of the line; the progressive shortening of the responses in the course of each experiment (see above and discussion later), which would have affected the later responses, to higher current intensities, more than the earlier ones, to lower current intensities, thus diminishing the slope of the line.

Discussion

The effects of tetra-ethylammonium iodide on "accommodation" and on threshold

Tetra-ethylammonium ions produce the same changes in frog nerve that removal of calcium ions produces. The experiments of Netter [1928], upon the action of various ions on the injury potential, indicate that the nerve membrane acts as an ionic sieve permeable only to cations below a certain size. He has concluded that the effective "mesh" of the sieve is affected little by changing the cations in the solution bathing the nerve. Netter found that neither tetra-ethylammonium ions nor calcium ions influenced the injury potential of frog nerve which had been previously soaked in glucose solution, and he has inferred that the nerve membrane is impermeable to these cations. Chemical studies also indicate that the nerve membrane is impermeable to calcium. Tipton [1934] has found that the calcium in nerve is present in a diffusible fraction, which he regards as present in the extracellular spaces, and an indiffusible fraction, which he regards as intracellular. We conclude, therefore, that the principal factor involved in the effects which we have studied is probably displacement by tetra-ethylammonium ions of calcium ions from the position that they normally occupy at the interface between the exterior of the nerve membrane and the surrounding solution.

Any more detailed explanation of the antagonism between calcium ions and tetra-ethylammonium ions necessitates answering two questions:

Why are the other cations of Ringer's solution, sodium and potassium, not involved in the same way that calcium is?

Why is tetra-ethylammonium the only symmetrical tetra-alkyl-ammonium cation that produces "spontaneous" activity?

An answer to the first of these is suggested by the work of Höber [1936] and of Wilbrandt [1937]. Since calcium ions and tetra-ethyl-ammonium ions are too large to penetrate the nerve membrane, and they are considerably more hydrophobic than sodium and potassium, their concentrations at the interface may well be greater than in the bulk of the surrounding solution. Increasing hydrophobic character in passing from lower to higher members of the series of tetra-alkylammonium ions [for estimates see Ing & Wright, 1931] must enter into the answer to the second question, but this factor alone cannot explain the uniqueness of the action of the tetra-ethylammonium ion. The other factor concerned is unknown. Extrapolation of conductivity data and calculation of the ionic radius in free solution by the Stokes-Einstein equation give

the values 2·78 Å. for the tetra-ethylammonium ion [Ing & Wright, 1933; Ing, 1936], and 3·0 Å. for the calcium ion [Landolt-Börnstein, 1927]. Whilst the approximate equality of these radii may be only a coincidence, it is possible that the other factor is size.

The effects of tetra-ethylammonium iodide on thresholds and on responses to constant current

Clearly, our findings involve more than a "reversal" of the normal electrotonic changes of excitability.

The dependence of the duration of repetitive response of nerve which had recently been treated with 30 millimolar solution upon current intensity, over a range of 2–7 times threshold, is a point of interest. Residual "accommodation" may have been one of the factors concerned in the ending of the response (and in the rarity with which "spontaneous" discharges occurred within a few seconds after the end of the response, if it be allowed that a part of a nerve remote from the point of stimulation can "accommodate" to its own action potential), although if any such "accommodation" were present in the quiescent intervals it was too small for us to measure. (Residual "accommodation" would hardly have been expected in view of our finding that the response of nerve which had been treated with 15 millimolar solution, to descending current, went on until break.) Probably a second factor contributing to the ending of the response and to the subsequent rarity of "spontaneous" discharges was a generalized failure of excitation ("refractoriness" or "fatiguability"). Such a failure, however, would not explain why, when the break threshold was below the make threshold, the duration of response at break of ascending current was shorter than that of the response at make with the same current descending. Local failure of excitation, which developed more rapidly at the anode following the break of current than at the cathode following the make of current, appears to have been the principal factor operating.

If it be assumed that for the response at make to be smaller when a current of a certain intensity is ascending nerve than when the current is descending indicates that impulses starting from the cathode, when more distal, are partly blocked in the anodic region, then in our experiments with Ringer-soaked preparations the independence of the size of muscle response of current direction indicates that the condition of the nerves was one in which currents of intensity up to seven times (make) threshold did not cause any block at the anode. Comparison of the responses given by the same preparations at make of descending and of

ascending current of 2–7 times threshold intensity after their nerves had been treated with 15 or with 30 millimolar solution indicates that then, however, the anode exerted a weak blocking action.

If an explanation similar to that above be assumed for the failure of preparations whose nerves had recently been treated with 30 millimolar solution to give responses with any but the highest intensities of descending current, it is to be concluded that an almost complete block was built up at the cathode in the few seconds which elapsed between make and break of current.

When the small irregularities which were superimposed on the earlier responses are taken into account it becomes evident that the local failures of excitation postulated in the three preceding paragraphs would have to be made up of two components: a depression (at the anode or at the cathode) developing smoothly after the application of current, and dependent upon the intensity of the current and upon the concentration of tetra-ethylammonium ions with which the nerve had been treated; a local prolongation of the relative refractory period at the point of stimulation. The transition to the intermittency and rapid failure exhibited in the later type of response would be accounted for by a progressive increase in the importance of the second factor.

The following observations by other workers suggest that certain of the effects of potassium ions are qualitatively similar to those of tetra-ethylammonium ions: the threshold for excitation at break of constant current was lowered relative to the threshold for excitation at make [Biedermann, 1898]; during the passage of small polarizing currents the threshold for excitation by superimposed long-lasting current was increased at the cathode and diminished in some, but not in all, instances at the anode [Chweitzer, 1935, 1937; Bouman, 1937]; during the passage of larger polarizing currents the threshold for superimposed long-lasting currents was increased at the anode and at the cathode [Chweitzer, 1935, 1937]. Should these apparent similarities in the effects of the two cations prove to be real they may be of interest on account of certain similarities in their chemical and physical properties.

Katz has recorded that treatment of nerve with sufficient sodium citrate to produce "spontaneous" activity reduced the rheobase to 1/8 to 1/10 of its initial value. In our experiments the action of tetra-ethylammonium ions on the electrotonic changes of excitability probably accounts for the smaller fall observed—1/4 to 1/8 of the initial value. So far as we know sodium citrate is without influence on the electrotonic changes of excitability of nerve.

SUMMARY

1. Treatment of frog nerve with Ringer's solution containing tetra-ethylammonium iodide (10 millimols per litre) produced a twofold change in the action potential: a prolongation of the negative after potential; a repetitive discharge in response to a single shock. Greater concentrations of tetra-ethylammonium iodide (15 millimols per litre and more) caused "spontaneous" asynchronous activity in nerve.

2. According to Hill's [1936] theory of excitation repetitive response in nerve is due to an increase in the time-constant of "accommodation" (λ) of the tissue. In these experiments this time-constant was measured and found to increase with increase in the concentration of tetra-ethylammonium iodide applied to the nerve.

3. Treatment of nerve with tetra-ethylammonium iodide-Ringer solution reduced the rheobase considerably below its normal value.

4. The "spontaneous" activity produced in nerve by tetra-ethyl-ammonium ions is the limiting result of lowering of the rheobase and an increase of the time-constant of "accommodation".

5. The changes in the time-constant of "accommodation" and rheobase could be prevented or reversed by an adequate concentration of calcium ions.

6. Tetra-ethylammonium ions reduced the threshold for excitation at break of constant current to a greater extent than the threshold for excitation at make. From examination of the responses of tetra-ethyl-ammonium iodide-treated nerve to constant currents 2–7 times make threshold it is inferred that local depressions of excitability were produced at the anode and at the cathode.

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EXCITATION AND INHIBITION IN THE OFF-EFFECT OF THE RETINA

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The retina differs from other sense organs by signalling cessation of stimulation with an off-effect, a new rise of positivity in the electroretinogram, and this phase of the retinal response is accompanied by a discharge of impulses through the optic nerve [Adrian & Matthews, 1927; Granit, 1933]. When a flash is thrown on the off-effect the latter is partly cut down by a large negative wave, first seen by Einthoven & Jolly [1908] and studied in detail by Granit & Riddell [1934] and Granit & Therman [1935] who showed that it was due to reactivation of the negative component P III of the retinal response (negative inhibitory P III) and was accompanied by inhibition, and a rapid diminution of the frequency of the impulses in the optic nerve. Here, therefore, was on record evidence to the effect that excitation and inhibition were accompanied by potentials of opposite sign in a structure belonging to the central nervous system (cf. Gasser's similar results with the spinal cord, e.g. Erlanger & Gasser [1937]), a problem later developed by Eccles [1936] in such an interesting manner with the aid of experiments on the superior cervical ganglion.

The whole off-effect could not, however, be removed in this way, suggesting that it might contain two components, one inhibitable, the other one non-inhibitable. A very important contribution to this problem was made by Hartline [1935] who recorded the impulses from individual fibres in the optic nerve and found that some of the fibres which discharged at the onset of stimulation also discharged at "off" whereas another group of fibres discharged *only* at cessation of stimulation. The discharge in the latter type of fibre was easily inhibited by re-illumination as with a flash on top of the off-effect. It is hardly necessary to emphasize how well these results harmonize with those just

reviewed. In addition they suggest that one part of the off-effect is more intimately connected with the positive excitatory component P II, another part with the negative inhibitory component P III of the retinal response.

We are now in a position to appreciate an observation made by Granit & Riddell [1934] which at that time was not clearly understood. After removal of the positive excitatory component P II of the electroretinogram the off-effect could be almost completely inhibited by a flash on top of the off-effect that remained. In such cases the electroretinogram was a practically pure negative potential rising rapidly towards the base line at "off". The flash momentarily re-established the negative potential reached during stimulation. In a normal electroretinogram only about 50 p.c. of the off-effect would have been removed. Why then this high degree of inhibition after removal of positive excitatory P II? Could it possibly mean that one part of the retinal off-effect is more intimately connected with the positive excitatory component P II and that this part had disappeared with the disappearance of P II? This fraction would then have to be non-inhibitable by a flash on the off-effect.

Below we have developed a method by means of which it is possible to remove in a simple manner an inhibitable as well as a non-inhibitable part of the off-effect. We shall show that the latter actually is more intimately connected with the positive component P II causing the initial *b*-wave of the electroretinogram, the former with the negative component P III.

Technique and procedure

The experiments were carried out with the same recording apparatus, used in several earlier contributions to similar problems: a string galvanometer and a directly coupled amplifier. Our preparation was the excised opened bulb of the frog, continuously illuminated with an adapting light, interrupted every now and then to give the off-effects to be experimented upon. From another source of light a $\frac{1}{10}$ sec. flash was made either to precede the off-effect or to fall just on top of it. The effect of this test light upon the off-effect has been studied. All experiments conform to this very simple type. Both lights fill the whole bulb.

Two different devices were used for the stimuli: (I) In a number of experiments spectral light from the Tutton monochromator, previously described [Granit & Munsterhjelm, 1937], illuminated the eye and the test-light flashes were delivered with a Compur shutter in the beam of a standard incandescent bulb made monochromatic with the aid of an Ilford "spectral filter". In such cases both lights were fitted to the

same spectral region. The Ilford "spectral filters" are made to cover relatively narrow spectral bands. We have not attempted to determine their transmission (see e.g. Wright [1937] for such measurements). We shall see below that our immediate concern is neither wave-length nor intensity as such, but relative size of *b*-wave and off-effect. (II) In another group of experiments a single light source was used, a Zeiss carbon arc with an electromagnetic device automatically regulating the distance between the carbons. By means of a block of prisms, arranged as described by Graham & Granit [1931], this light was divided into two separate equally bright beams which were united again to fall on the opened eye of the frog. Tscherning neutral tint filters were placed in the beams to give the relative levels of intensity wanted for adapting light and test light. In most experiments the full strength of the arc was used for the $\frac{1}{10}$ sec. flash with the eye adapted to 1/100th of this intensity. However, other levels and differences of intensity also were tested.

In order to reproduce the results to be described it is not necessary to use particularly high intensities. In fact, we shall see later that too strong flashes (test lights) introduce various complications. Nor is it necessary to limit the observations to any particular state of adaptation. But it is advisable to use an intensity above which the off-effect does not fluctuate. We generally started with a dark-adapted eye, left for 5 min. in the adapting light before beginning the experiment. This light was interrupted about once in a minute to give the off-effect to be experimented upon.

Results

"Refractoriness" of off-effect to a preceding b-wave

If part of the off-effect be due to elements reacting with a positive initial *b*-wave of P II, that part should show the same kind of "refractoriness" to a previous *b*-wave as would be evident when one *b*-wave follows immediately after another. Fig. 1 shows diagrammatically what happens when a number of identical flashes follow one another at identical intervals. The diagram is based on the work of Piper [1911] and of Creed & Granit [1933] who showed that it takes some time before, in a series of flashes (flicker), the individual small positive *b*-waves can break through the large *b*-wave caused by the first flash. The elements reacting with positive P II are, as it were, in a state of refractoriness caused by the first flash. The term "refractoriness" is throughout this work used in a purely descriptive sense.

Accordingly, if part of the off-effect is due to positive P II in elements capable of reacting with a *b*-wave, that part should disappear when a

b-wave is made to precede the off-effect. The latter should diminish. In order to test this proposition the $\frac{1}{10}$ sec. flash is thrown in about 200–500 msec. before the off-effect is about to take place. The flash must be made strong enough to give a definite *b*-wave when superimposed upon the steady potential kept up by the permanent adapting illumination. In Fig. 2, A shows the off-effect control, B the *b*-wave elicited by

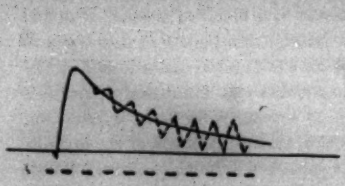

Fig. 1.

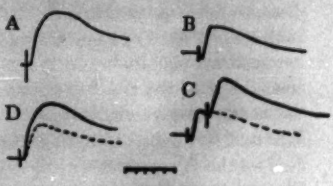

Fig. 2.

Fig. 1. Diagram showing the effect of later flashes as compared with the initial *a*- and *b*-waves caused by the first flash of the series.

Fig. 2. A, off-effect alone, off-control; B, preceding *b*-wave alone, *b*-control; C, combined effect of preceding *b*-wave *plus* off-effect, broken line showing course of *b*-control; D, off-control drawn in full; off-remainder, as reconstructed from curve C, shown by broken line. Time interval, 200 msec.

the flash superimposed upon the adapting light. When the flash a few minutes later is thrown in about half a second before the adapting light is interrupted to give the off-effect, record C is obtained. The latter is a combination of the preceding *b*-wave *plus* off-effect. From this record we have to subtract the amount of potential that the *b*-wave would have given alone, that is, curve B, the *b*-wave control. The course of the latter change is shown by the broken line in the same figure, curve C. The residual off-effect is replotted in curve D below the off-effect control. The broken line shows the reduced off-effect. Apparently the preceding *b*-wave has succeeded in removing about 40 p.c. of the off-effect.

The off-effect therefore is not independent of the potential change of the same sign known as a *b*-wave. It is, in fact, partly influenced by a preceding *b*-wave as if that part of the off-effect itself had been a *b*-wave. Questions of relative intensity and interval between *b*-wave and off-effect will temporarily be left aside while we continue to follow the main trend of the experimental analysis. In the experiments to follow the $\frac{1}{10}$ sec. flash will be put in within about 500 msec. before the adapting light is turned out to give rise to the off-effect.

The inhibitable part of the off-effect

In Fig. 3, A shows the *b*-wave elicited by the $\frac{1}{10}$ sec. flash superimposed upon the adapting light. The same flash is then thrown in on top of the off-effect, giving the record B, in which the off-effect control is drawn in broken lines. There is now to be seen the typical negative notch, which, as we know, is accompanied by inhibition in the optic nerve. To what part of the off-effect does this negative notch belong? Is it still there after removal of that part of the off-effect which above was found to be influenced by a preceding *b*-wave?

An answer to these questions may be obtained by the simple expedient of throwing the flash in twice, first some 200–500 msec. before the off-effect is about to take place and then a second time on top of the off-effect. The

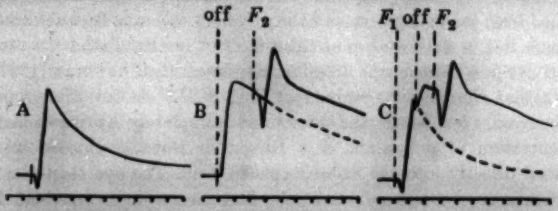

Fig. 3. A, *b*-wave control given by preceding flash F_1 alone; B, off-control with flash F_2 superimposed on top of it; off-control continued as broken line; C, combined effect of F_1 *plus* off-effect *plus* F_2, broken line showing course of *b*-wave control. Time interval, 200 msec.

record thus obtained is shown as C in Fig. 3. The off-effect has been cut down by the preceding flash as shown by the flash control drawn in broken lines in the record. The inhibitory notch is as big as in curve B where the flash is falling on the off-effect control. The amount of inhibition, measured as negative P III, has therefore not been influenced by the removal of a large fraction of the off-effect. The inhibitable part of the off-effect is constant. This presupposes that the flash on the off-effect is properly timed [Granit & Riddell, 1934].

The difference between the flash control and the off-effect potential in curve C shows the amount of off-potential left by the preceding flash. If the inhibitory notch had been touching the broken line it would have meant complete momentary removal of the off-effect. However, there is a fraction left, about 12 p.c., which the two flashes together have not succeeded in removing. How much is left depends upon the relative size of off-effect and flash as well as upon state of adaptation. The inhibitory

notch is small or absent in a completely dark-adapted eye, stimulated for a short time with a high intensity [Granit & Riddell, 1934]. If the flash is very strong, relative to the size of the off-effect, it causes a relatively larger positive *b*-wave after the negative phase. This *b*-wave interrupts the negative notch at some point not directly obtainable from the record. Therefore the lowest point on the negative notch need not necessarily represent the maximum amount of inhibitory P III elicited under given conditions. It merely represents the point of balance between two opposite potentials affecting the same measuring instrument. In order to obtain the negative notch alone it is necessary first to remove the positive component P II of the electroretinogram.

This was done by Granit & Riddell [1934] who found—as has already been mentioned—that, when the electroretinogram after massaging the eye had been made negative and the off-effect was a swing-back towards the base line, a flash on top of this off-effect re-established the state of negativity prevalent during illumination. Recently Therman [1937] has shown that the positive component P II of the electroretinogram can be removed even better and sometimes completely by increasing the concentration of potassium in a Ringer or isotonic glucose solution pipetted directly into the excised opened bulb. The eye then reacts to illumination with a very brisk and large negative component P III swinging rapidly back towards the base line at "off". In this eye *the whole off-effect is inhibitable.* A flash causes a negative notch reaching the level of negativity from which the off-effect rose. A flash, thrown in just before the off-effect is about to appear, elicits no positive response, only a small negative *a*-wave. It has no influence on the off-effect of the type studied in the previous section. Thus, *when the b-wave due to positive P II disappears, so also does that part of the off-effect (non-inhibitable off) which showed refractoriness to b-waves, and the remaining part of the off-effect is completely inhibitable.*

Conclusion

We have on purpose left out all complications in order first to present the main results of this investigation into the nature of the off-effect. These are that the off-effect consists of at least two elements, parts or components: (I) One is part and parcel of the positive component P II, responsible for the *b*-wave, and disappears when it falls into the wake of refractoriness left by a preceding *b*-wave. After removal of P II it likewise disappears. This we call the *non-inhibitable off-effect.* (II) The other one does not require the positive component P II and is not in a similar manner influenced by a preceding *b*-wave. Only the negative

component P III is needed for this element of the off-effect to appear. It reacts with a deep and rapid negative wave ("negative notch" of Granit & Riddell [1934] when a flash is thrown on top of it. This negative notch coincides with an inhibition of the discharge in the optic nerve [Granit & Therman, 1935]. After removal of all positive reactions to light by treating the retina with a sufficiently strong solution of $K^{\cdot}$-ions [Therman, 1937] it is found that the flash on the off-effect re-establishes the level of negativity reached by P III during illumination. On account of these properties this part of the off-effect has been called the *inhibitable off-effect*.

The inhibitable and non-inhibitable retinal off-effects probably are retinal equivalents to fibres with similar properties [Hartline, 1935]. The fibres reacting only or chiefly at "off" stop their discharge upon re-illumination of the eye. Our results indicate that during illumination the corresponding retinal elements develop negative P III thereby preparing them for the discharge that takes place at "off". The fibres reacting at both "on" and "off" probably are connected with the elements responsible for the non-inhibitable off-effect which is so sensitive to a preceding *b*-wave.

Complicating factors

Under this heading we are collecting a number of observations which, though incapable of invalidating the main line of arguments presented above, yet show that we must reckon with phenomena of interference between the components of the electroretinogram as complicating factors. Thus, for instance, we shall show that strong preceding *b*-waves not only remove part of the off-effect—non-inhibitable off—but also may modify the remainder.

In the first instance one would naturally expect a strong preceding *b*-wave to remove a greater fraction of the off-effect than a weak one. Within limits this is so. The *b*-wave caused by the preceding flash may be diminished by either reducing the intensity of the stimulus or by shortening its duration. In both cases the off-effect emerges less and less influenced by the flash. This therefore is according to expectation. But unexpected changes begin to make their appearance when the preceding *b*-waves are made larger than the off-effects. This, of course, means that the light eliciting the *b*-wave is a great deal stronger than the adapting light upon which it is superimposed. In order not to have to use excessively strong stimuli it is advisable to adapt the eye to an intensity which is only just sufficiently strong to give an off-effect at about 0·10–0·20 mV.

Fig. 4 shows a typical example. The eye is continuously illuminated by wave-length 0·650 μ from the monochromator and the interruption of this light gives an off-effect control varying somewhat in size as shown by the several lines of curve A. It is perhaps unnecessarily small, 0·10 mV. Curve B illustrates the flash control, curve C the combination

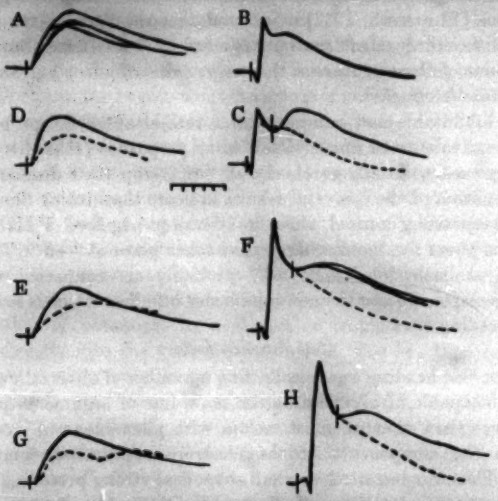

Fig. 4. A, a number of off-controls; B, *b*-wave control (Note: diphasic *b*-wave); C, combined effect of *b*-wave *plus* off-effect, course of *b*-wave control shown by broken line; D, average off-control drawn above off-remainder reconstructed from C. The pairs E and F, G and H correspond to the pair D and C, the preceding flash in the two latter pairs having been increased in strength as shown by bigger *b*-wave control drawn in broken lines in F and H. Note: with increasing strength of preceding flash the off-remainder becomes diphasic. Time interval, 200 msec.

flash plus off-effect, and curve D the reduced off-effect plotted below the average "off" control. The Ilford spectral red is used for the flash. So far the figure is similar to Fig. 1. Curves E and F show a similar set of records from the same experiment when, somewhat later, the *b*-wave has been made bigger by increasing the strength of the flash. The two records E and F correspond to C and D, F being the combined effect of *b*-wave plus off-effect with the *b*-wave control shown in broken lines, E the off-effect control with the reduced off-effect remainder in broken lines. The off-effect

control is now about 45 p.c. of the preceding *b*-wave. Despite this the percentage reduction is roughly constant, that is, the amount of non-inhibitable "off" is not influenced by the size of the preceding *b*-wave when it is increased beyond a certain limit. But the rate of rise of the remainder is reduced and there is an indication of a second phase rising above the level of the control. A further increase in the intensity of the flash does not make the *b*-wave very much bigger but definitely splits up the reduced off-effect into two phases as shown by the records G and H.

Sometimes the influence of a strong preceding *b*-wave on the off-effect is even more striking. The *b*-wave and off-effect controls may be reasonably normal and yet, when the *b*-wave is made to precede the off-effect, the string suddenly stops dead at a certain plateau level of positivity where

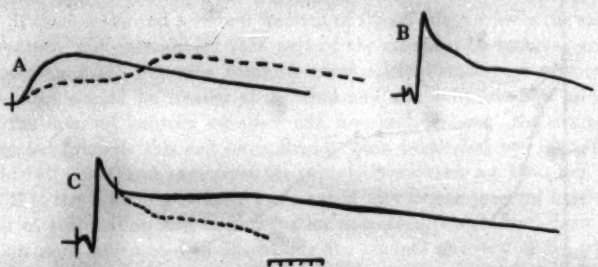

Fig. 5. B, *b*-wave control; A, off-effect control drawn in full, off-remainder as reconstructed from C shown in broken lines; C, combined effect of preceding flash *plus* off-effect. Time interval, 200 msec.

it remains for a considerable time. The off-effect is so much changed that one has the impression that the preceding *b*-wave somehow has succeeded in activating a new slow element of positivity of the kind that one sometimes sees when recording from the retina with localized electrodes [Granit, 1937]. Some specimens are shown in Fig. 5. At the moment we have no definite explanation of these phenomena to offer.

The *b*-waves of Fig. 4 (F and H) are interesting inasmuch as there is a small rise of positivity before the *a*-wave changes into the positive phase. A similar positive phase preceding the *a*-wave has previously been seen by Granit & Riddell [1934] and recently by Jolly [1936]. We take this opportunity to point out that initial irregularities of this fast type are often seen when for some reason or other retinal detachment has taken place with consequent formation of a small fold suitably localized relative to the electrodes.

Interval between preceding flash and off-effect

When trying to work out curves illustrating the effect of the interval between the preceding flash and the off-effect on the amount of off-effect removed we first believed that the curve had an optimum at certain short intervals between 250 and 500 msec. But later we came to the conclusion that such optima only were apparent. To begin with, off-effects tend to vary in size more than *b*-waves, thereby simulating irregular optima in the curve. But in most eyes there is in addition a slow change taking place during the time the retina is continuously illuminated. This effect is illustrated in Fig. 6.

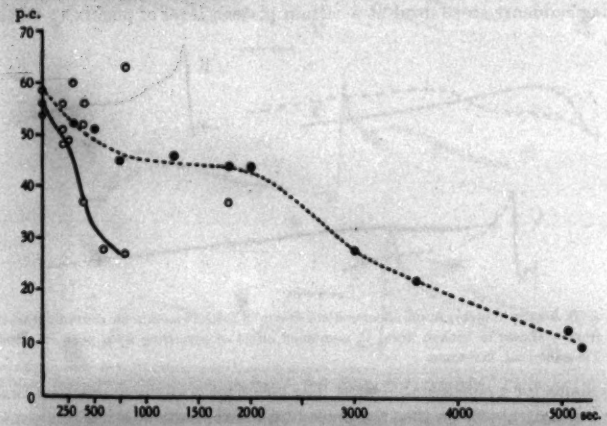

Fig. 6. Ordinates: percentage off-effect removed by preceding flash. Abscissae: interval between flash and off-effect. Circles, values obtained early in the experiment; filled circles, later values.

The interval between preceding flash and off-effect is shown on the abscissa, the removed percentage of the off-effect on the ordinate. In order to secure as many observations as are plotted in this figure one may have to keep the eye illuminated for a couple of hours. During this period the time constants of the retinal reaction gradually alter. The flash succeeds in influencing the off-effect at increasingly longer intervals. For this reason it was found advisable to divide the values into two groups: those obtained in the beginning of the experiment which in the figure are given as open circles, and later values that are given as filled circles.

Towards the end of the experiment the influence of the preceding flash on the off-effect is traceable for 5–6 sec.

The removed percentage of the off-effect has been calculated in the same way as above: the *b*-wave caused by the flash control has been subtracted from the combined curve, flash plus off-effect, and the off-effect remainder compared with the "off" control. The difference in height between "off" control and "off" remainder has been given in percentage of the former. We have also tried other ways of plotting and estimating the amount removed aiming at including the fact that at short intervals the off-effect is delayed. But the method adopted in Fig. 6 was found to present the results in the simplest and most straightforward manner.

Off-effect preceding b-wave

If the *b*-wave and a certain fraction of the off-effect arise in the same structure, not only should that part of the off-effect be removed by a preceding flash, but vice versa, *b*-waves elicited during or after the off-effect should be smaller than usual and gradually increase in size as the interval between off-effect and *b*-wave lengthens. Experiments directed towards this end have already been performed by Granit & Riddell [1934] and have given the expected result (see e.g. their Fig. 10, p. 15): the *b*-wave gradually increases in size as the interval between end of stimulation and re-illumination increases. The curves given by them were interpreted as illustrating the gradual recovery of the component P II, an interpretation which is still true in the light of our present knowledge. However, they did not realize that part of the positive P II belonged to the (non-inhibitable) off-effect, as the present results indicate, but referred the whole recovery to a recovery from the depression of positivity at the end of illumination. Probably both factors enter into play: the *b*-waves on top of the off-effect are small both because the off-effect has used or is utilizing some of the elements reacting with *b*-waves of the rapid type [Granit & Munsterhjelm, 1937] as well as because of a gradual recovery of all the *b*-elements from the after-effects of illumination.

Discussion

We are aware of the need for some modification of the analysis of the frog's retinal action potential as given schematically by Granit & Riddell [1934] in order to include the results presented in this paper. However, we shall not attempt to introduce the necessary modifications now. The analysis is still good enough as a diagram illustrating in a general way the course of the three fundamental components of the

retinal response. Since it was published the multiple nature of the *b*-wave and the off-effect [Chaffee *et al.* 1923; Granit & Munsterhjelm, 1937] has been definitely established and we now obtain in this laboratory with localized electrodes slow and fast *b*-waves and off-effects in routine experiments with the electroretinogram. The important work of Hartline [1935] and the results presented in this paper have to be kept in mind when the details of the analysis at some later stage are filled in.

In this discussion we only want to draw attention to two aspects of our results. One is the connexion with the phenomena of "rebound" and inhibition in the central nervous system, especially interesting in view of the dual nature of the off-effect. As a first approximation one is bound to believe that the difference between the inhibitable and non-inhibitable off-components is quantitative rather than qualitative. Some structures, the most inhibitable ones, have in a higher degree than others a capacity for charging themselves with inhibitory P III when stimulated with light. Without being too definite with regard to the nature of the inhibitory block one can assume this blocking mechanism to be more or less highly developed or to act directly or indirectly upon the locus where the impulses are generated. The retinal results do not in the first instance support the view that the inhibitory mechanism could be identified with the subnormal phase and positive after-potential of peripheral nerve [see Gasser, e.g. in Erlanger & Gasser, 1937; Eccles, 1936]. Inhibition in the retina seems to be independent of excitation and is best developed when there is least reason to assume that impulses have been passing through the structures concerned.

Another point worth emphasizing here is the general usefulness of this procedure with one flash before and an identical flash just on top of the off-effect in the analysis of the properties of the off-effect, when a large number of variables such as wave-length, intensity, area, state of adaptation, etc. have to be studied. The method has already been successfully applied by one of us (P. O. T.) in an analysis of the influence of a number of biologically active substances on the electrical response of the eye. We have suggested that the inhibitable and non-inhibitable retinal off-effects have their equivalents in corresponding fibres of the optic nerve, studied by Hartline [1935]. If this be so—as we have every reason to believe—our method will become an easy and valuable substitute to the exceedingly laborious dissection of individual fibres in the nerve, whenever, after introduction of any of the above mentioned variables, the relative distribution of inhibitable and non-inhibitable elements in the off-effect has to be measured. The advantage of precision,

offered by the unitary analysis, is in such cases hardly compensated for by the labour involved, there being in addition some risk for chance factors like size and toughness of the fibres determining the end result.

Summary

The retinal electrical responses of excised, opened frogs' eyes have been recorded with the aid of a string galvanometer and a directly coupled amplifier.

The retina has been kept illuminated by one light, every now and then interrupted to give an off-effect. A $\frac{1}{10}$ sec. flash from another lamp has been made to precede the interruption of the adapting light or to fall just on top of the off-effect.

When the flash precedes the off-effect the latter is reduced by a certain amount, dependent upon the relative strength of the two stimuli, indicating that some of the elements reacting at "off" also had responded to the flash and therefore were incapable of reacting with an off-effect immediately after the flash had been delivered.

The flash falling on top of the off-effect produces the well-known negative "notch", that coincides with inhibition in the optic nerve. The depth of this inhibitory notch is independent of whether a flash has *preceded* the off-effect or not. This is held to signify that the elements reacting to re-illumination with inhibition are independent and not identical with those that could be put out of function by a preceding flash.

The significance of the interval between flash and off-effect as well as certain complications arising when the flashes are very strong are illustrated by suitably designed experiments.

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THE ORIGIN AND LIMITS OF THE SPECIFIC DYNAMIC ACTION OF INTRAVENOUS GLUCOSE[1]

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In an earlier paper the laws of the utilization of intravenous glucose, infused constantly at rates of 1–9 g./kg./hr., were described [Wierzuchowski, 1936]. In a following publication glucose oxidation under the same conditions was shown and correlated with other kinds of glucose removal from the blood [Wierzuchowski, 1937]. In the present paper are discussed the heat production obtained by indirect calorimetry, and especially the excess heat production over the basal level (specific dynamic action = S.D.A.) with all other metabolic processes observed in the present work during the continuous intravenous glucose supply at various rates. The methods used are exactly the same as in the two above-mentioned publications. In fact, they all refer to the same experiments and to the same animals. The technique was briefly as follows:

In two well-trained dogs (Ordo and Andro) glucose was injected by vein during 6 hr. at a constant rate in various experiments, from 1 up to 9 g. of glucose per kg. body weight per hr. Respiratory metabolism was examined at 26° C. by the open circuit method with the help of gas analysis, before, during and after infusion, until the S.D.A. disappeared. In addition, urine N, blood and urine sugar, blood and urine lactic acid, relative hæmoglobin concentration and urine volume were determined at frequent intervals. Heart and respiration rate and body temperature were checked at each determination of the respiratory exchange. The results were worked out by the planimetric interpolation method and the errors evaluated. The excess heat was calculated for each experiment

[1] The preliminary communications of this work were published in the *Summaries of the Communications of the XIV Congress of Polish Medicine and Natural History* in Poznań, September 1933; later in *C.R. Soc. Biol., Paris* (1935), 119, 436, 664.

separately, assuming as basis the initial basal metabolism in the same experiment. One protocol is given in Fig. 10 as an example of how the work was done.

RESULTS

The results will be divided into three groups: A, basal values; B, totality of the S.D.A. during entire experiment; C, S.D.A. at the peak of glucose action at each infusion rate.

A. Basal values

It is evident from Table I that during a 15–18 months' observation period the body weight in both dogs varied, on the average, ±1·32 p.c. The ano-nasal length, necessary for the Cowgill & Drabkin [1927] formula, was measured by means of wooden callipers in the right- and left-side position. The index of nutrition [Cowgill & Drabkin] was close to 0·3 in both dogs and varied on the average only ±0·51 p.c., indicating a medium state of nutrition.

Table I. Some basal values obtained in two normal female dogs used in the present work. M.-R. = Meeh-Rubner; C. & D. = Cowgill & Drabkin

	Dog Andro (during 15 months)			Dog Ordo (during 18 months)		
Value measured	Average value	Absolute deviation from the basal	Standard percentage deviation from the basal	Average value	Absolute deviation from the basal	Standard percentage deviation from the basal
Number of experiments	22	—	—	19	—	—
Number of determinations	43	—	—	46	—	—
Ano-nasal length (cm.)	84·60	—	—	73·56	—	—
Body weight (kg.)	17·35	± 0·23	± 1·33	10·57	± 0·14	± 1·32
Index of nutrition (C. & D.)	0·3059	± 0·0013	± 0·42	0·2982	± 0·0018	± 0·60
Rectal temperature (° C.)	38·8	± 0·3	± 0·77	38·6	± 0·2	± 0·52
Lung ventilation (c.c./kg./min.)	175·4	±14·6	± 8·33	189·8	±11·0	± 5·79
Number of respirations per min.	12·5	± 1·6	±12·8	10·8	± 0·9	± 8·33
Surface area (M.-R.) (sq. m.)	0·751	± 0·008	± 1·07	0·539	± 0·006	± 1·19
Surface area (C. & D.) (sq. m.)	0·688	± 0·004	± 0·58	0·499	± 0·004	± 0·78
Heat production (cal./hr.)	29·50	± 1·23	± 4·15	18·32	± 0·97	± 5·28
Heat production (cal./kg./hr.)	1·701	—	—	1·734	—	—
Basal heat (cal./sq. m. (M.-R.) per 24 hr.)	939·4	±37·8	± 4·02	814·1	±38·4	± 4·72
Basal heat (cal./sq.m. (C. & D.) per 24 hr.)	1025·5	±41·1	± 4·01	883·0	±41·0	± 4·64
Total R.Q.	0·723	± 0·009	± 1·25	0·721	± 0·016	± 2·22
Non-protein R.Q.	0·712	± 0·011	± 1·55	0·707	± 0·018	± 2·55
Last basal taken after the last food intake	22 hr. 14 min.	—	—	24 hr. 11 min.	—	—
Urine N (g./hr.)	0·1225	± 0·0305	±24·9	0·0914	± 0·0164	±18·0
Urine N (mg./kg./hr.)	7·00	—	—	8·66	—	—
Heat from protein (cal./hr.)	3·18	—	—	2·37	—	—
Heat from fat (cal./hr.)	25·76	—	—	15·95	—	—
Heat from carbohydrate (cal./hr.)	0·56	—	—	—	—	—

The average body temperature measured in the cleaned rectum amounted to 38·7° C. with variation of ±0·3° in Andro and ±0·2° in Ordo. Lanfranchi [1933] gives 38·4° as the average normal temperature of a dog. The temperature of our dogs seems to have been slightly raised during the experiment, but it must be remembered that they had a catheter inserted in the bladder during the whole experiment, and that 3–4 hr. before the basal determinations two skin incisions were made for the cannula and for blood sampling (Table I).

The average lung ventilation was 182·6 c.c./kg./min., with a variation of ±7 p.c. The number of respirations was 11·7, with variation of ±10·6 p.c.

The surface area was calculated by means of two formulae. In the formula of Meeh-Rubner the constant 0·112 of Rubner was used. This has been applied for years by Lusk, and recently, after careful scrutiny, again recommended for adult dogs by Benedict [1934]. The surface area in two dogs, calculated from the Meeh-Rubner formula, varied on an average by ±1·13 p.c. and in one from the Cowgill & Drabkin formula, by ±0·68 p.c.

The basal heat per sq. metre was larger in the bigger animal. The absolute values were: by the Meeh-Rubner formula, 939·4 cal./sq. m./24 hr. for the larger dog, and 814·1 cal. for the smaller one; whereas the normal average in the female dogs with untouched body coverings amounted to 772 cal. in the work of Lusk & Du Bois [1924]. By the Cowgill & Drabkin formula the value for the larger dog was 1025·5 cal. and for the smaller 883·0 cal./sq.m./24 hr. Both values are higher than the standard value given by Cowgill & Drabkin for female dogs: 836 cal.

The basal metabolism of our two dogs was therefore on the upper limit of the normal. This was owing to the slight rise of the average body temperature over the usual normal, as may be concluded from Fig. 1, in which the basal heat is plotted against body temperature. The height of the basal heat was in direct proportion to the body temperature. It is easy to deduce from the graph that at a temperature of 38·4° C., which they should show under perfectly normal conditions, our dogs have a heat production of about 800 cal., somewhat similar to that shown by Lusk's dogs (772 cal.). The standard day-to-day deviation of the basal metabolism was ±4 p.c. in the larger, and ±4·7 p.c. in the smaller dog. In the literature the most constant values obtained in the dog are those on Lusk's dog XIX [1931] in which, during periods of several months in two successive years, the variation was ±2·9 p.c.

During the two to four basal tests, performed at the end of periods of 24 hr. fasting, the total R.Q. dropped slightly, reaching in one dog in the last determination (or determinations) the level of 0·723, with a standard deviation of ±1·25 p.c., and in the other, 0·721 with a deviation of ±2·22 p.c. After subtraction of the protein respiratory exchange the average non-protein R.Q. was, in one dog, 0·712, and in the other, 0·707. Practically the only foodstuff then burnt besides the protein was fat. Even if the fat level of oxidation was not exactly reached in the last basal period, there was usually an interval of 1 hr. or more between it and the moment of glucose infusion, during which the non-protein R.Q.

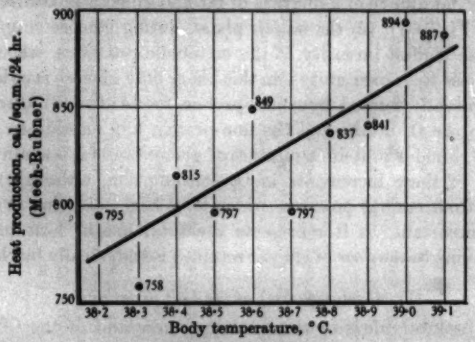

Fig. 1. Basal heat production of two dogs in connexion with body temperature. Mean of eighty-six determinations.

could still reach the fat level exactly, as may be seen in the first 10 min. of glucose infusion [Wierzuchowski, 1937]. The fat level of non-protein oxidation gives a convenient background for the observation of glucose combustion, which enters the metabolism as a new quantity, as it were, and not as an augmentation of the already existing carbohydrate oxidation. We do not see any better check for our technique than these basal non-protein R.Q.'s representing pure fat oxidation during fasting, seeing that they are obtained under the conditions of the experiment itself.

Nitrogen was eliminated in urine of the larger dog at a speed of 7·0 mg./kg./hr., with a variation of ±25 p.c., and in the smaller, of 8·6 mg./kg./hr., with a deviation of ±18 p.c. This amount corresponds, in the larger dog, with 3·18 cal., and in the smaller with 2·37 cal., indicating that under basal conditions about 11·9 p.c. of the heat was derived from protein and the rest almost exclusively from fat.

It seems justifiable, therefore, to assume that the present data are consistent enough to serve as a basis for special experiments undertaken to test the influence of intravenous glucose on heat formation and conducted over long periods.

B. S.D.A. IN THE WHOLE EXPERIMENT

With glucose infusion of 6 hr. duration there is a rise of O_2 consumption over the basal rate, and three periods may be differentiated therein: (1) the *initial* one, during glucose infusion, in which the non-protein R.Q. rises from the fat level to the carbohydrate level, and in which first fat and then a mixture of fat and glucose is oxidized [Wierzuchowski, 1937]; (2) the *middle* phase, during glucose supply, representing the highest intensity of the metabolic processes, when the R.Q. remains close to or over unity—in this phase only glucose is oxidized and fat is eventually formed from non-protein foodstuffs; (3) *after infusion* of glucose the O_2 intake and the non-protein R.Q. subside to the basal value and, besides protein, a mixture of glucose and fat is again oxidized. The sum of these increments in O_2 consumption, although evidently resulting from various processes which have been occurring at a varying rate, is important, as it represents the total rise of heat production corresponding to the dose of glucose retained metabolically by the tissues.

(1) *Absolute values of the heat distribution*

The absolute values of the total O_2 intake and of the CO_2 output calculated per sq. metre of the body surface per hour for the entire experiment (Tables II and III) show that the increase of these values over basal, under the influence of glucose infusion during 6 hr., lasted 8–17 hr. (including the infusion period). These values are presented here for comparison with those in other individuals and other species in accordance with Dryer's conclusion [Orr-Ewing, 1931]. The largest *increase* of O_2 consumption per sq. metre over basal is about 5 l./hr. and of CO_2 elimination about 7·5 l./hr. For both *increases* at higher rates of supply there is a limiting level per unit of surface just as there is per unit of mass [Wierzuchowski, 1937]. The limiting level of the gaseous exchange cannot be observed on the absolute values contained in Tables II and III, but only on the increases of the gaseous exchange over basal, which are easily deduced from these tables.

The rise of body temperature contributed to some extent to the rise of O_2 consumption. The body temperature rose during glucose supply to a point which was, on the average, +0·78° C. above the initial value, and

TABLE II. O_2 consumption per surface area (Cowgill & Drabkin) during continuous glucose infusion at a rate of 23–192 g./sq.m./hr. (=1–9 g./kg./hr.). Mean of twenty-nine experiments in two dogs. Values prepared for comparison with other species

Rate of glucose supply		Litres of O_2 consumption/sq.m./hr.															
			Glucose infusion														
g./sq.m./hr.	g./kg./hr.	Basal	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	7 hr.	8 hr.	9 hr.	10 hr.	11 hr.	12 hr.	13 hr.	14 hr.	15 hr.	17 hr.
22·86	1	8·235	8·976	9·404	8·974	9·196	9·461	9·315	8·592	8·518	8·420	8·378	8·048	8·196	8·270	—	—	—
45·61	2	7·898	9·962	10·080	10·309	10·157	10·183	10·000	9·397	8·445	7·809	7·470	7·282	7·384	7·467	—	—	—
69·02	3	7·863	9·571	10·660	11·220	11·370	11·380	11·070	10·170	8·790	7·869	7·507	7·319	7·507	7·617	—	—	—
93·80	4	8·872	10·931	13·320	12·370	12·410	12·340	12·340	11·450	9·894	8·839	8·428	8·167	8·217	8·265	—	—	—
116·50	5	8·703	10·926	13·250	12·910	13·150	12·820	13·130	11·540	10·101	9·202	8·890	8·632	8·452	8·498	—	—	—
139·00	6	8·580	11·015	13·310	13·560	13·230	12·910	12·720	12·140	10·761	9·724	9·127	9·122	8·852	8·587	—	—	—
162·80	7	8·588	10·539	13·450	13·510	13·530	12·870	12·570	11·404	10·315	9·565	9·252	8·908	8·719	8·610	—	—	—
186·50	8	9·310	11·100	13·790	14·060	14·300	13·350	12·790	12·070	11·070	10·370	9·804	9·295	8·799	8·459	—	—	—
191·60*	9	8·419	10·950	13·200	12·950	12·500	12·020	11·820	11·020	9·886	9·216	9·209	9·120	8·961	8·780	8·639	8·508	8·338

* One dog only (Ordo).

TABLE III. CO_2 elimination per surface area (Cowgill & Drabkin) during continuous intravenous glucose supply at a rate of 23–192 g./sq.m./hr. (=1 to 9 g./kg./hr.). Mean of twenty-nine experiments in two dogs. For comparison with values obtained with other species

Rate of glucose supply		Litres of CO_2 elimination/sq.m./hr.															
			Glucose infusion														
g./sq.m./hr.	g./kg./hr.	Basal	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	7 hr.	8 hr.	9 hr.	10 hr.	11 hr.	12 hr.	13 hr.	14 hr.	15 hr.	16 hr.
22·86	1	6·079	7·189	8·718	8·482	9·131	9·216	8·923	7·759	7·232	6·830	6·532	6·304	6·199	6·144	—	—	—
45·61	2	5·874	8·209	9·840	10·067	10·105	10·244	9·795	8·918	7·787	7·040	6·629	6·354	6·283	6·248	—	—	—
69·02	3	5·738	7·917	10·354	11·070	11·310	11·300	10·771	10·172	8·624	7·529	6·969	6·623	6·702	6·773	—	—	—
93·80	4	6·461	9·422	13·265	12·695	12·770	12·450	12·480	11·495	9·802	8·582	8·065	7·706	7·541	7·421	—	—	—
116·50	5	6·361	9·200	13·120	12·930	13·430	13·140	13·560	11·740	10·018	8·941	8·508	8·183	7·946	7·786	—	—	—
139·00	6	6·302	9·322	12·980	13·690	13·560	13·370	13·110	12·740	10·925	9·719	9·327	8·982	8·553	8·140	—	—	—
162·80	7	6·206	9·047	13·560	13·845	13·145	13·205	12·870	11·944	10·488	9·434	8·959	8·655	8·294	8·202	—	—	—
186·50	8	6·560	9·501	13·440	14·020	14·390	13·350	12·830	12·090	10·891	10·059	9·477	8·993	8·317	7·827	—	—	—
191·60*	9	5·963	9·362	13·210	12·900	12·740	12·170	12·150	11·350	10·066	9·252	9·220	9·036	8·567	8·182	7·987	7·794	7·545

* For one dog only (Ordo).

then, after infusion, declined on the average by −0·61° C. below the initial level. The rise of temperature was similar for all the injection rates, so that this may be considered as a more or less constant factor by which the heat production and the S.D.A. should be diminished. The final decline of the O_2 intake below the basal level should be ascribed to depression of body temperature.

In Fig. 2 only the parts of the experiments, including the infusion period, were considered, during which a rise of heat over basal was present. Each gram of glucose per kg. introduced during 6 hr. of infusion furnished material containing 22·46 cal. The heat of glucose assimilated in this period, compared with that supplied, shows that only at the 1 g. rate of supply per kg./hr. is there hardly any wastage of glucose in the urine. When the rate of supply increases, increasing amounts of glucose escape the metabolic removal, being excreted in the urine [Wierzuchowski, 1936]. Finally, at the 7 g. rate of infusion (157·2 cal.), 90·1 cal. are assimilated. This last value persists at the 8 g. rate (91·96 cal.), showing the limit of assimilation. A value for the 9 g. rate was obtained in one dog only, during an unusually long period (17 hr., Tables II and III) in which renal damage appeared, so that glucose retention resulted with a consequently longer utilization of glucose and a longer duration of the S.D.A. There is only very slight wastage of glucose in the form of lactic acid (Fig. 2) excreted in the urine.

Of the material representing 22·43 cal., assimilated at the 1 g. rate of glucose supply, one-half is oxidized, the other half being transformed in a non-oxidative way (Fig. 2). If we subtract from the last value the heat of glucose turned (1) into fat by decarboxylation (calculated from the respiratory exchange after Lusk [cf. Wierzuchowski & Ling, 1925]), and (2) into lactic acid excreted in the urine, the heat of glucose "stored" is obtained, the values of which are shown in brackets in Fig. 2. The large percentage of the heat of glucose oxidized at the 1 g. rate of supply is, however, exceptional over the whole range of glucose infusion rates [Wierzuchowski, 1937]. When the speed of supply increases the calories of glucose stored grow much quicker than those of oxidized glucose. The former reach practically the limit at the 7 g. rate of supply—61·48 cal., the latter at the 5 g. rate—about 25 cal. At the 9 g. rate, as mentioned above, the conditions are special. Also the heat of glucose transformed into fat reaches its limiting value at about the 6 g. rate of infusion (Fig. 2, upper part). As the loss of lactic acid in the urine constantly increases with the increasing rate of glucose injection, it does not reach a limit [see Wierzuchowski, 1937].

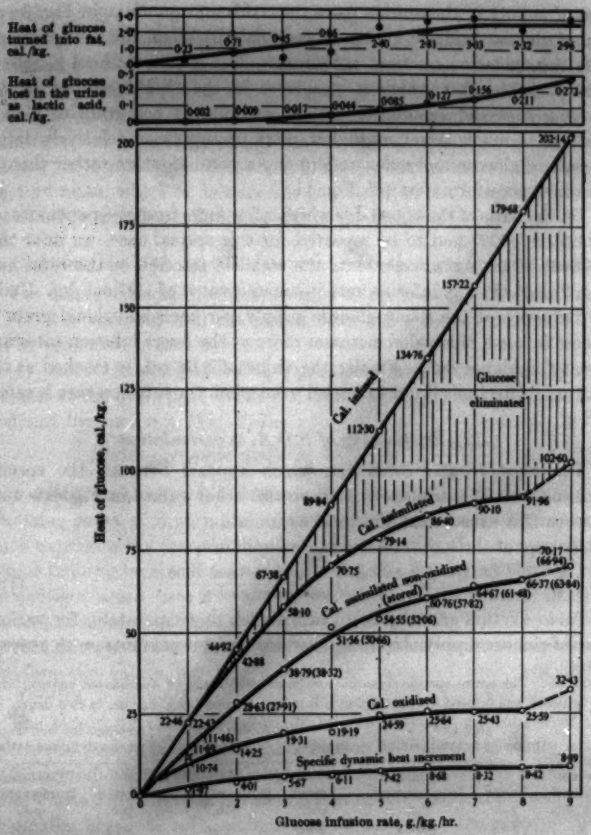
Heat of glucose turned into fat, cal./kg.
Heat of glucose lost in the urine as lactic acid, cal./kg.
Heat of glucose, cal./kg.
Glucose infusion rate, g./kg./hr.
Cal. injected
Glucose eliminated
Cal. assimilated
Cal. assimilated non-oxidised (stored)
Cal. oxidised
Specific dynamic heat increment

Fig. 2. Distribution of the heat during the S.D.A. of glucose introduced by vein at various constant rates. Mean of twenty-nine experiments in two dogs. Experiment with the 9 g. infusion rate obtained in one dog only (exceptionally long observation period). Values concern the *entire duration* of the S.D.A.

The exceptional position of the heat of glucose oxidized at the 1 g. rate of supply is also visible from the oxidation quotient in Fig. 3: per 1 g. of glucose oxidized 1·94 g. is assimilated. When the rate of supply rises the oxidation quotient amounts to 3·02, then, at the 4 g. rate, it rises to 3·74, after which it vacillates in the neighbourhood of 3·5, showing that the amount of glucose assimilated per gram of glucose oxidized is much larger at higher rates of supply, a relatively larger amount of glucose molecules undergoing a non-oxidative rather than an oxidative transformation (cf. Fig. 11).

On the basis of the above, for which a few facts from the paper already published [1937] had to be repeated for this special case, we infer that the heat of the S.D.A. represents the smallest fraction of the total heat (Fig. 2). At the 1 g. infusion rate it shows a value of 1·97 cal./kg. Under the influence of the rising glucose supply per each additional gram of glucose infused, this value increases more at the lower infusion rates and less at the higher ones. Finally the value of 8·68 cal. is reached at the 6 g. rate. This limit is not exceeded even when the infusion rate is raised to 8 g.

(2) *Relationship of S.D.A. to assimilation*

The most regular connexion which appears between the specific dynamic heat of intravenous glucose and other values in Fig. 2 is that between this excess heat and total assimilation:

Percentage of the S.D.A. referred to assimilation

$$= \frac{\text{total heat increase over basal} \times 100}{\text{heat of assimilated glucose}} \qquad \ldots\ldots(1)$$

In Table IV this quantity is given for each dog separately, for various rates of glucose supply. In the smaller dog Ordo, it amounts on an average

TABLE IV. The entire specific dynamic action as percentage of assimilated energy, when glucose was infused during 6 hr. at a constant rate of 1–9 g./kg./hr. in two dogs.

Rate of glucose infusion g./kg./hr.	Dog Ordo Specific dynamic action		Dog Andro Specific dynamic action		Average for two dogs Specific dynamic action	
	Found p.c.	Deviation from average p.c.	Found p.c.	Deviation from average p.c.	Found p.c.	Deviation from average p.c.
1	8·93	+0·33	8·65	−1·35	8·79	−0·51
2	8·27	−0·33	10·47	+0·47	9·37	+0·07
3	9·01	+0·41	10·54	+0·54	9·77	+0·47
4	8·04	−0·56	9·17	−0·83	8·61	−0·69
5	8·91	+0·31	9·84	−0·16	9·37	+0·07
6	9·67	+1·07	10·43	+0·43	10·05	+0·75
7	7·88	−0·72	10·53	+0·53	9·21	−0·09
8	8·03	−0·57	10·37	+0·37	9·20	−0·10
9	8·65	+0·05	—	—	—	—
Average	8·60	±0·48	10·00	±0·59	9·30	±0·34

to 8·60 p.c. of the heat content of the assimilated material, with a standard deviation of ±0·48 p.c., maximum +1·07, minimum −0·72 p.c. In the larger dog Andro this relationship to assimilation revealed the value of 10·00 p.c., ±0·59 p.c., maximum +0·54, minimum −1·35 p.c. The average in 2 dogs is (Table IV) 9·30 p.c., ±0·34 p.c., maximum +0·75, minimum −0·69 p.c. This is the average expenditure of energy necessary for intravenous glucose assimilation as compared with the total material utilized. It is strikingly constant (cf. Fig. 4) within the whole range of injection rates, which is, besides the constancy of the basal non-protein R.Q. of 0·709, another indirect check on our technique.

(3) *Relationship of the specific dynamic heat to the heat of glucose oxidized*

The next relationship which we may settle from the absolute values in Fig. 2 is that between the excess heat and glucose oxidation. This will be presented in two forms:

(*a*) *Oxido-dynamic quotient.* Let us call the ratio of the heat of glucose oxidized at the same time as the S.D.A. lasted (Fig. 2), to the excess heat produced by the S.D.A. (Fig. 2) the

$$\text{Oxido-dynamic quotient} = \frac{\text{cal. of glucose oxidized}}{\text{cal. of the excess heat}} \quad \ldots\ldots(2)$$

It amounts, for the 1 g. infusion rate, to 5·94 (Fig. 3). It declines with increasing rates of supply and comes to a standstill with higher rates which expresses the fact that the two original series of values reach the highest limiting level and remain there (Fig. 2). Finally, at the 9 g. rate the quotient again rises a little, corresponding to the excessive rise of the heat of glucose oxidized, which is explained by the fact that because of renal damage the S.D.A. of retained glucose lasted much longer and therefore the values per kg., without the factor of time, are higher (cf. Fig. 10).

The reason for the high value of the oxido-dynamic quotient at the 1 g. rate of supply is that glucose oxidation at this rate not only raises the heat production over basal, but also entirely replaces fat in the non-protein metabolic mixture. As the further relative increases of oxidation are smaller than the relative increases of the S.D.A., the ratio of these values must decrease for the increasing rates of supply. It will be shown later on (Table IX) that above the 1 g. rate of infusion the increments of heat take place at the expense of glucose oxidation. The oxido-dynamic quotient for the entire course of the S.D.A. indicates that there is a three- to six-fold excess of the oxidative energy from glucose to cover the specific dynamic loss of heat.

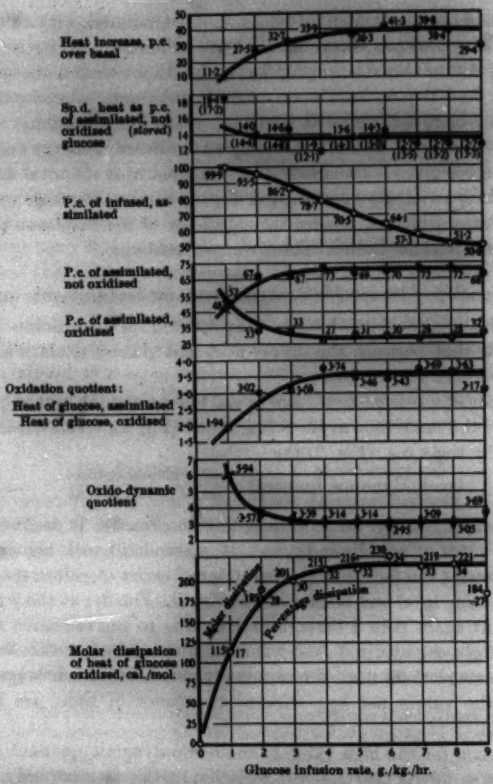
Heat increase, p.c. over basal
S.p.d. heat as p.c. of assimilated, not oxidised (stored) glucose
P.c. of infused, assimilated
P.c. of assimilated, not oxidised
P.c. of assimilated, oxidised
Oxidation quotient: Heat of glucose, assimilated / Heat of glucose, oxidised
Oxido-dynamic quotient
Molar dissipation of heat of glucose oxidised, cal./mol.
Molar dissipation
Percentage dissipation
Glucose infusion rate, g./kg./hr.

Fig. 3. Various metabolic relationships during the time in which the S.D.A. took place, in connexion with constant intravenous supply of glucose. Based on values in Fig. 2. Values of stored glucose in brackets.

(*b*) *Molar dissipation of heat of oxidizing glucose.* This value is interesting for thermochemical considerations. The formula

$$\text{Molar dissipation of heat} = \frac{\text{cal. of the excess heat} \times 180}{\text{g. of glucose oxidized}}, \quad \ldots\ldots(3)$$

expresses in cal./mol. of glucose oxidized how many calories of the excess heat appear when 1 mol. of glucose is being burnt in the body. If

we take the extreme values from Fig. 3 we see that at the 1 g. rate of supply 115 cal./mol. are liberated, i.e. 17 p.c. (Fig. 3) of the energy content of 1 mol. (673·8 cal.). With the growing rate of glucose infusion this value increases and attains, at the 6 g. rate, 230 cal./mol., which is about 34 p.c. of the energy content of 1 mol. The dissipation is then doubled.

This percentage of the loss of the oxidative energy of glucose in the form of S.D.A. may be expressed by the following simple formula:

Percentage of the S.D.A. referred to glucose oxidation

$$= \frac{\text{cal. of the excess heat} \times 100}{\text{cal. of glucose oxidized}}, \qquad \text{.....(4)}$$

and will be the object of Discussion (part III) and of Table VIII.

These relationships, considered for the total S.D.A. of injected glucose, show that there is no constant connexion between the specific dynamic heat of intravenous glucose and glucose oxidation.

(4) *Excess heat and the non-oxidative utilization of glucose*

This relationship is represented by:

Percentage of the S.D.A. referred to the non-oxidized part of assimilated glucose

$$= \frac{\text{cal. of the excess heat} \times 100}{(\text{total cal. assimilated} - \text{cal. of glucose oxidized})}. \qquad \text{.....(5)}$$

It amounts, for the 1 g. rate of glucose supply, to 18 p.c., and for all other rates, to 12–14 p.c. (Fig. 3). This regularity, although not quite satisfactory, is noteworthy.

The following value, which will be used later in the Discussion, is a more correct one:

Percentage of the S.D.A. referred to storage of glucose

$$= \frac{\text{cal. of the excess heat} \times 100}{(\text{cal. gluc. assim.} - \text{cal. gluc. oxid.} - \text{cal. gluc. to fat} - \text{cal. gluc. elim. lact. acid})}.$$

.....(6)

In this expression the values determined by formula (5) are purified by the elimination, in the denominator, from the total cal. of glucose assimilated, of not only the cal. of glucose oxidized but also of the cal. of glucose converted into fat (Fig. 2) and of the cal. of glucose eliminated in the urine as lactic acid (Fig. 2). Although the quantities in the denominator (Fig. 2, in brackets) represent chiefly glycogen formation

from glucose injected, they also represent the phosphorylated part of glucose, the alleged masked fat formation from this hexose and some other intermediary reactions, which are brought into play. The S.D.A., calculated according to formula (6), amounts to 12–17 p.c. (Fig. 3, in brackets, also in Table VIII). It is therefore quite close to that determined by formula (5).

(5) *Heat increase over basal during excess heat production*

This is the average heat increase during the entire period in which the S.D.A. was present. It is slight for the 1 g. rate (11 p.c.) (Fig. 3), then rises, when the supply increases, and reaches its highest level at the 6 g. rate (41 p.c.). It remains at this level for the 7 and 8 g. rate, and decreases for the 9 g. rate, owing to the above-mentioned special conditions of these experiments. All these values were calculated according to the formula

Percentage of the S.D.A. referred to the basal metabolism

$$= \left(\frac{\text{total heat during S.D.A.} \times 100}{\text{basal heat during S.D.A.}}\right) - 100. \qquad \ldots\ldots(7)$$

C. S.D.A. AT THE CULMINATION OF ASSIMILATIVE AND OXIDATIVE GLUCOSE REMOVAL

The part of the S.D.A. between the fourth and sixth hour of infusion is important, because of the highest intensity of glucose oxidation in this period at each infusion rate. If there are any connexions between the S.D.A. of glucose and the oxidation of this foodstuff, they ought to appear in this phase of the experiment, this period having the additional advantage that the metabolic conditions in it are fixed at a definite level, which must be considered as characteristic for each given rate of glucose inflow.

(1) *Heat distribution*

Table V shows the absolute increase of heat over the basal value with various rates of glucose supply, calculated not only per unit of mass (as in the former section), but also per unit of time. It amounts, at the 1 g. rate of glucose supply, to 0·32 cal./kg./hr. When the speed of glucose inflow increases, it rises at a progressively slower rate, the highest value reached being 1·10 cal./kg./hr. The limit is practically attained at the 6 g. rate of injection.

Table V gives a review of the energetic values obtained in the present work at the peak of glucose action. The values of total glucose assimilation, calculated in cal. [Wierzuchowski, 1936] and of glucose oxidation

TABLE V. Heat distribution during fourth to sixth hour of constant intravenous infusion of glucose from 1 to 9 g./kg./hr. The values of respiratory exchange and of blood chemistry taken from two dogs (twenty-nine experiments); the values of assimilation from the same two dogs + two additional dogs (together forty-eight experiments)

Glucose infusion rate g./kg./hr.	Heat of glucose injected cal./kg./hr.	Total average heat output cal./kg./hr.	Heat of the S.D.A. cal./kg./hr.	Heat of glucose assimilated cal./kg./hr.	Heat of glucose oxidized cal./kg./hr.	Heat of glucose assimilated, non-oxidized cal./kg./hr.	Heat of glucose stored cal./kg./hr.	Heat of glucose turned into fat cal./kg./hr.	Heat of glucose lost as urine lactic acid cal./kg./hr.	Heat of glucose stagnating as lactic acid in the body fluids cal./kg./hr.	Blood lactic acid (6 hr. of infusion) mg./100 c.c.	Blood sugar (fourth to sixth hr. of injection) mg./100 c.c.
1	3·74	2·02	0·32	3·90	1·74	2·17	2·10	0·07	0	0·06	3·7	151
2	7·49	2·20	0·58	7·09	1·94	5·15	5·00	0·15	0·001	0·26	13·3	258
3	11·23	2·45	0·85	9·51	2·27	7·24	7·17	0·07	0·002	0·33	15·6	368
4	14·97	2·62	0·85	11·14	2·32	8·82	8·43	0·39	0·007	0·37	18·5	659
5	18·72	2·65	1·04	12·28	2·54	9·74	9·29	0·45	0·013	0·54	23·2	645
6	22·46	2·79	1·08	11·37	2·57	8·80	8·33	0·47	0·019	0·52	25·9	961
7	26·20	2·80	1·10	11·99	2·63	9·36	9·00	0·36	0·023	0·60	30·7	1430
8	29·94	2·90	1·09	11·09	2·64	8·45	8·15	0·30	0·030	0·64	30·9	1842
9	33·68	2·81	0·97	Kidney lesion	2·59	Kidney lesion	—	0·39	0·039	0·67	30·8	2130

TABLE VI. Fourth to sixth hour of glucose infusion at various constant rates. Relationships of the S.D.A. to other metabolic activities, calculated from Table V, for two dogs

Rate of glucose supply g./kg./hr.	S.D.A. as p.c. of heat of glucose assimilated (formula (1))	S.D.A. as p.c. of heat of glucose oxidized (formula (4))	S.D.A. as p.c. of heat of glucose assimilated, non-oxidized (formula (5))	S.D.A. as p.c. of heat of glucose stored (formula (6))	Molar dissipation of heat of oxidized glucose (formula (3)) cal./mol.	Oxido-dynamic quotient (formula (2))	Increase of heat over basal (formula (7)) p.c.	Glucose oxidation as p.c. of assimilation	Glucose non-oxidized as p.c. of total glucose assimilated
1	8·22	18·66	14·80	15·24	126	5·60	19·3	43·4	55·6
2	8·23	30·05	11·34	11·60	203	3·33	36·1	27·4	72·6
3	8·89	37·21	11·68	11·85	251	2·69	52·9	23·9	76·1
4	7·62	36·81	9·62	10·08	248	2·79	48·3	20·8	79·2
5	8·47	41·19	10·66	11·19	278	2·44	59·8	20·7	79·3
6	9·51	42·14	12·28	12·96	284	2·44	63·4	22·6	77·4
7	9·15	41·71	11·73	12·23	281	2·41	64·8	22·0	78·0
8	9·86	41·37	12·93	13·38	279	2·44	61·4	23·8	76·3
9	—	37·30*	—	—	251*	2·70*	52·7	—	—

* For one dog only. Values of assimilation cannot be calculated for this rate of supply.

[Wierzuchowski, 1937] expressed as heat, are quoted from the former publications. The derived values of the heat of glucose assimilated, non-oxidized, and of glucose stored were obtained in the same manner as in the former section. Most of these quantities grow with the increase of the speed of injection, and at the 5 g. rate attain the highest limiting level, which amounts, for total assimilation, to 12·28 cal./kg./hr., i.e. 7 times more than the basal heat production (average for two dogs: 1·718 cal./kg./hr.). Of this energy 2·54 cal. appear immediately in the form of heat of glucose oxidized, 0·45 cal. is turned into fat, 0·01 cal. split into lactic acid and eliminated in the urine, and the rest, 9·29 cal., are deposited chiefly as glycogen.

(2) *Relation of excess heat to glucose assimilation*

In this period the excess heat is 8·74 p.c. of the total energy content of glucose assimilated, with ±0·61 p.c. deviation (Tables VI and VIII). The slight increase shown in this value, as the speed of supply rises, may not be real because the total assimilation at this stage of the experiments was calculated by means of a formula, and this may have given slightly too low values for total assimilation at higher speeds of supply. We find again, then, that the excess heat is in direct proportion to the assimilation, so that it remains fairly constant within the whole range of infusion and assimilation rates.

(3) *Excess heat and glucose oxidation*

It is evident from Table VI that the relationship of excess heat to glucose oxidation is not constant, but changes with the infusion rate. The molar dissipation of heat from burning glucose, which at the 1 g. infusion rate is 126 cal./mol. of oxidized glucose, increases with the increasing inflow of glucose: at the 2 g. rate it becomes 203 cal./mol., at the 3 g. rate 251 cal. and at the 5 g. rate almost 280 cal., and this level persists at still higher rates. This means that at the 1 g. rate of supply 19 p.c. of the energy of glucose oxidized is used to cover the specific dynamic loss of heat, and at the highest rates this increases to 41 or 42 p.c. (Table VI).

The same is indicated by the oxido-dynamic quotient, which shows how many cal. are produced by glucose oxidation, when 1 cal. is lost in the form of S.D.A. This ratio amounts to 5·6 at the 1 g. rate, then decreases with the rising introduction of glucose, and at the 5 g. rate the value 2·44 is reached, and this is maintained at higher rates of glucose supply (Table VI). Table VI, if compared with Fig. 3, shows, for the

oxido-dynamic quotient, somewhat lower and for molar dissipation, somewhat higher values. The reason for this is that in the entire period during which the S.D.A. appears, the post-infusion part (Fig. 3) contributed much to oxidation and little to the S.D.A. Owing to this fact the calculated relationships changed accordingly.

(4) *Excess heat production and non-oxidative glucose utilization*

If we reckon (see formula 5) the specific dynamic heat as a percentage of the heat of glucose assimilated, non-oxidized (Table VI), we obtain, at the 1 g. rate of infusion, a slightly higher value (14·8 p.c.) than at the other rates of introduction (9·6–12·3 p.c.). This shows a certain regularity, similar to that observed for the total heat increase (Fig. 3). If we calculate the same relationship for the heat of glucose stored, according to formula (6), the values are similar (Tables VI and VIII).

(5) *Heat increment over basal*

In this period of the highest intensity of glucose transformation the highest relative rise of heat production over basal is observed in connexion with various rates of glucose administration (Table VI) (formula (7)). From the low value at the 1 g. rate of injection (19·3 p.c.) it rises until, at the rate of 6 g./kg./hr., it attains 64·8 p.c., the highest obtained in the dog during excessive glucose saturation of its body at rest.

DISCUSSION SUPPLEMENTED BY SOME ADDITIONAL RESULTS

The author wishes to connect the series of papers published lately in this *Journal* with his former work. All this work has been done on female dogs kept under constant conditions of diet, of temperature, and of life in general. Sugar solutions, always in the same concentration, were introduced under identical conditions by vein at a constant rate over a number of hours. Grafe [1934], as one of the most authoritative reviewers of this question, wishes to retain the term S.D.A. only for phenomena of heat production originating from food given by mouth. Perhaps the mechanism of the S.D.A. of sugar given by mouth and by vein is somewhat different. The information, however, won by constant infusion is more exact, as the supply of sugar to the cells is known for each fraction of time, and lends itself more to quantitative consideration.

Let us now consider whether the various facts observed in the present work may reveal the nature of the S.D.A. of glucose. This consideration will be presented under the following headings, which express

the relationship of the excess heat production to: (i) assimilation; (ii) fat formation; (iii) glucose oxidation; (iv) storage of glucose; (v) glycolysis; (vi) blood sugar level; and (vii) kidney function.

(i) *S.D.A. of glucose and the entire utilization of this hexose*

It is an important fact that the S.D.A. of intravenous glucose within the whole range of administration rates is strictly proportional to the transformative removal of glucose (Fig. 4). This applies not only to the

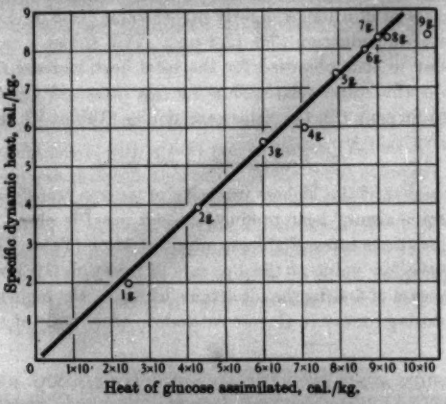

Fig. 4. Twelve hours' period. Proportionality between the excess heat over basal and the heat of assimilated glucose in two dogs, twenty-nine experiments. The figure close to each point denotes the rate of glucose infusion. Value for the 9 g. rate obtained in one dog only.

figure of a whole experiment (Table IV) but also to that part of it in which the phenomena of utilization were at their highest (fourth to sixth hour of glucose supply) (Table VI). Therefore the same is probably also true for each point of the experiment, each mol. of glucose retained liberating, under these conditions, an average of 62·7 cal., irrespective of all other factors. The law may be expressed thus:

$$\frac{\text{cal. of S.D.A.}}{\text{cal. of assimilated glucose}} = \text{constant} = 0{\cdot}093,$$

i.e. each cal. of assimilated glucose conditions the liberation of 0·093 cal. of excess heat.

As a result of the increasing supply of glucose solution augmenting the volume of circulating blood the heart rate increases by about 5·7 beats per min. (Fig. 5) per 1 g./kg./hr. addition of glucose, i.e. per 5 c.c. addition of glucose solution per kg./hr. This must raise the utilization of blood glucose proportionally with the blood glucose level [Evans *et al.* 1935]. We have encountered no limit to the increase of heart rate at the higher infusion rates. As a contracting muscle the heart obeys quite different laws of glucose utilization from those obeyed by resting muscle.

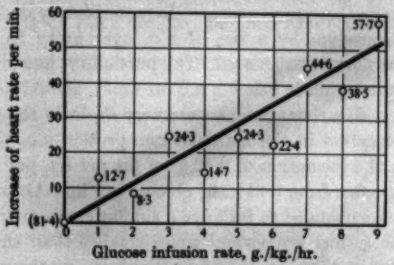

Fig. 5. Heart rate and the speed of infusion of 20 p.c. glucose solution, expressed in g./kg./hr. Dog Ordo, seventeen experiments, 120 readings.

From what has just been said and from the following synopsis (Table VII) it is clear that the relation of S.D.A. to assimilated sugar is almost constant for the tested mono- and disaccharides, introduced

TABLE VII. Review of author's work on the specific dynamic action of sugars administered by vein at constant rate of 2 g./kg./hr. during a certain number of hours, in dogs

Year of publication	Experimental conditions	Material supplied	Duration of infusion hr.	Specific dynamic action as p.c. of assimilation	Heat of oxidizing sugar dissipated as S.D.A. cal./mol.	Lactic acid increase in the blood mg./100 c.c.	Fat formation per whole experiment g.
1931	Normal dogs and carbohydrate diet	Glucose	3	9·7	214	11	—
		Fructose	3	13·8*	278*	32	—
1934	Amytal anæsthesia and sugar diet	Glucose	6	9·7	202	15	—
		Fructose	6	10·2	185	44	—
		Maltose	6	9·1	182	18	—
1935	Protein diet	Fructose	10	11·0	218	24	3·3
	Sugar diet	,,	10	12·0	220	32	11·8
	10 days' fast	,,	10	10·9	233	57	0·4

* Fructose slightly toxic.

intravenously. Under manifold conditions (Table VII) glucose had the same S.D.A., when referred to assimilation, as in the present experiments. Fructose gave a slightly higher value. Maltose showed the same value as glucose.

(ii) *Excess heat and the oxido-reduction processes (fat formation)*

Chemically the fat formation from glucose may proceed either by the condensation of pyruvic acid [Smedley-MacLean, 1936] or by the reactions described by Krebs [1936]. From the standpoint of the respiratory exchange these processes are characterized by decarboxylations manifesting themselves by a rise of R.Q. over unity from which the formation of fat may be calculated. The possibility, however, of masked fat formation taking place below a R.Q. of unity, should not be ignored.

The manifest decarboxylations were very slight in the present work, expressing themselves in the maximal non-protein R.Q. of 1·05, which corresponds to the formation of 0·30 g. of fat per kg. per whole experiment, and originates from 0·81 g. of glucose per kg. Although in Fig. 2 the cal. of glucose changed to fat have a similar curve as the excess heat production, the former are several times smaller than the latter. The connexion between them is, therefore, unlikely, as the process would produce more energy than is contained in the substances concerned (Fig. 11).

There is also a number of other arguments which speaks against fat formation as the source of S.D.A. Wierzuchowski & Ling [1925] showed on a growing hog in which enormous quantities of fat were formed from carbohydrate, that there was no relationship between the rise of R.Q. over unity and the heat increase over basal. Moreover, these oxido-reduction processes were only slightly exothermic, 5 p.c. of the energy of glucose converted into fat being set free in the process, which is in agreement with the work of Terroine & Bonnet on *Sterigmatocystis nigra*, discussed in 1929 (10 p.c. energy loss). Furthermore, it was shown by the author (Table VII, 1935) that during the utilization of fructose under varying conditions its S.D.A., as referred to assimilation, was similar, irrespective of the large or negligible formation of fat from this furanose.

Moreover, if the present work is considered, the fat formation seems to be connected with those metabolic functions which appear to be less concerned with the S.D.A., and, vice versa, the fat formation seems to have less contact with those activities which do play a part in the origin of S.D.A. The fact that fat formation occurs only in a highly

oxygenated medium in yeast and during excessive oxidation of carbohydrate in higher animals, e.g. the hog, suggests that the oxidation of glucose and its oxido-reduction must be fairly closely connected (Fig. 11). On the contrary, recent work on the whole animal [Schur *et al.* 1934] and on isolated fat tissue [Henle & Szpingier, 1936] argues in favour of direct transformation of glucose into fat, without the intermediary of glycogen.

(iii) *Excess heat production and glucose oxidation*

There is a regularity between the excess heat and glucose oxidation within the limits of 1–8 g./kg./hr., but not between zero and the 1 g.

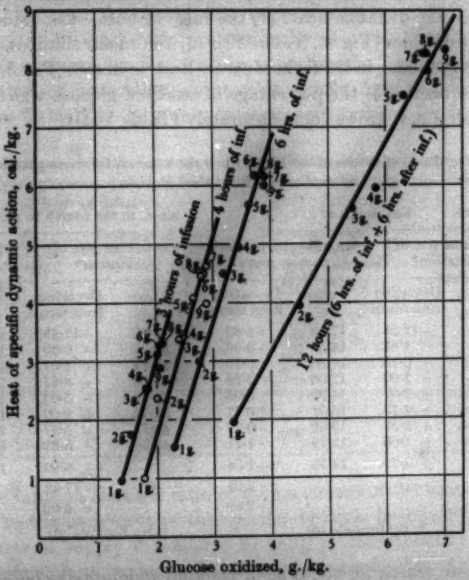
Heat of specific dynamic action, cal./kg.
Glucose oxidized, g./kg.
2 hours of infusion
4 hours of inf.
6 hrs. of inf.
1½ hours (6 hrs. of inf. + 6 hrs. after inf.)

Fig. 6. Relationship of excess heat production to glucose oxidation, the latter being expressed per whole period indicated, in various phases of the experiments. Twenty-nine experiments in two dogs. Glucose infusion rate indicated at each dot.

rate of supply (Fig. 6). Heat increase, plotted against grams of glucose oxidized, gives, for various periods of the experiments, a straight or almost straight line. These curves do not, however, pass as straight lines

through the zero point of the co-ordinate system, as there is obviously some other law ruling below the rate of supply of 1 g./kg./hr. No matter how we tried to correlate the heat increase and the glucose oxidation, they did not agree within the whole range of infusion rates (Fig. 11). As the excess heat production is in constant proportion to the total assimilation, the S.D.A. can be proportional to the glucose oxidation only if the part oxidized is a constant fraction of the total assimilated. This is not the case, for when the rate of supply increases, the percentage of glucose oxidized diminishes, and the percentage of glucose removed by the tissues in a non-oxidative way increases (Fig. 3, Table VI). Hence there is a change in all the values expressing the relationship of glucose oxidation heat to specific dynamic heat: (1) the ratio of both—the oxido-dynamic quotient—declines (Fig. 3, Table VI), (2) the molar dissipation of heat of oxidizing glucose, in the form of excess heat, increases (Fig. 3, TableVI), and consequently (3) the percentage of oxidized glucose which is lost in the form of S.D.A. grows commensurately (Table VIII).

TABLE VIII. Magnitude of standard deviation when the S.D.A. is taken as percentage of the heat of oxidized and of stored glucose

Glucose infusion rate g./kg./hr.	Entire S.D.A.				S.D.A. in the fourth to sixth hour of supply			
	S.D.A. as p.c. of oxidation*		S.D.A. as p.c. of heat of glucose stored†		S.D.A. as p.c. of oxidation*		S.D.A. as p.c. of heat of glucose stored†	
	Value p.c.	Deviation from mean	Value p.c.	Deviation from mean	Value p.c.	Deviation from mean	Value p.c.	Deviation from mean
1	17·06	−12·86	17·19	+2·89	18·66	−17·48	15·24	+2·92
2	28·07	− 1·85	14·36	+0·06	30·05	+ 6·09	11·60	−0·72
3	29·77	− 0·15	14·80	+0·50	37·21	+ 1·07	11·85	−0·47
4	31·92	+ 2·00	12·06	−2·24	36·81	+ 0·67	10·08	−2·34
5	31·99	+ 2·07	14·25	−0·05	41·19	+ 5·05	11·19	−1·13
6	34·10	+ 4·18	15·01	+0·71	42·14	+ 6·00	12·96	+0·64
7	32·50	+ 2·58	13·53	−0·77	41·71	+ 5·57	12·23	−0·09
8	33·98	+ 4·06	13·19	−1·11	41·37	+ 5·23	13·38	+1·06
Average	29·92	± 3·72	14·30	±1·04		± 5·89	12·32	+1·17
Minimum				−2·24		−17·48		−2·34
Maximum				+2·89		+ 6·09		+2·92

* By formula (4). † By formula (6).

When we search for the type of reaction which might be responsible for the S.D.A., we must remember that this reaction should give, when referred to the excess heat, a percentage deviation at least as small as that given by the excess heat when referred to the model reaction—the entire assimilation (9·30 p.c. ± 0·34 p.c.). Let us first test, from this standpoint, the heat of glucose oxidized (Table VIII). If considered for the entire period during which the S.D.A. lasted, the average excess heat

is 29·92 p.c. of the heat of glucose oxidized synchronously with the standard deviation of ±3·72 p.c., minimum −12·86 p.c., maximum +4·18 p.c. (Table VIII). If the culmination period only is analysed (fourth to sixth hour of infusion) this value amounts to 36·14 p.c., ±5·89, minimum −17·48, maximum +6·09 p.c. It is clear from this that glucose oxidation, considered as the origin of the S.D.A., does not show so great a regularity as that offered by reference to total assimilation.

As already mentioned, when, under the influence of glucose supply, the R.Q. of unity has once been reached, each addition of glucose can raise glucose oxidation only by increasing at the same time the O_2 intake. Therefore, between the 1 and 9 g. rates of supply, each increase of glucose oxidation must raise proportionally also the specific dynamic heat. This is shown by Fig. 6. The value for the 1 g. rate of glucose supply is out of proportion, as appears clearly from the incremental relations in Table IX. It is only between zero and the 1 g./kg./hr. rate

TABLE IX. Comparison of the increments of the oxidative and specific dynamic heat per one gram of glucose supplied in addition to various pre-existing infusion rates, in two dogs, during 6 hr. of infusion.

Increase of glucose supply between the rates g./kg./hr.	Increase of the oxidative heat per g. increase of glucose supply cal.	Increase of the specific dynamic heat per g. increase of glucose supply cal.	Ratio: Increase of oxidative heat / Increase of specific dynamic heat cal.
0–1	1·447	0·254	5·70
1–2	0·259	0·245	1·06
2–3	0·264	0·248	1·06
3–4	0·167	0·077	2·17
4–5	0·128	0·119	1·08
5–6	0·056	0·085	0·66
6–7	0·106	−0·006	0
7–8	−0·025	0·008	0
8–9	0·032	−0·031	0

of glucose infusion that the ratio of the increment of the heat of glucose oxidized to the increment of the specific dynamic heat is 5·7. For the higher rates of supply it amounts to unity or thereabouts. From the 6 g. rate upwards the values either do not increase further or show negative quantities. If by mass action of glucose its oxidation is chiefly meant, the part of the experiment between the 1 and 6 g./kg./hr. rate of glucose supply does indeed follow to some extent the mass action law.

Besides the quantitative disagreement between increase of heat production and synchronous glucose oxidation there are also some discrepancies in time between them. In the *first* 10 min. of glucose infusion

O_2 intake rises without rise of the R.Q. [Wierzuchowski, 1937]. Towards the *end* of the S.D.A. there is no more increase of heat production over basal; nevertheless the R.Q. still remains high over the initial value, in spite of the fact that the blood sugar has dropped to the initial level (cf. Table X). These two discordances may be explained on the basis of

TABLE X. Blood-sugar level, non-protein R.Q. and heat production at the beginning, at the culmination, and at the end of the specific dynamic action of intravenous glucose infused at various rates. Dog Andro

			Basal values			Sixth hour of glucose infusion			Seventh hour after glucose infusion		
Exp. no.	Date	Glucose infused rate g./kg./hr.	Heat cal.	Non-protein R.Q.	Blood sugar mg./100 c.c.	Heat cal.	Non-protein R.Q.	Blood sugar* mg./100 c.c.	Heat cal.	Non-protein R.Q.	Blood sugar mg./100 c.c.
7	30. xi. 1931	1	31·07	0·706	94	34·37	0·963	152	31·18	0·714	94
3	20. x. 1931	2	27·14	0·720	99	38·43	1·019	266	27·61	0·821	101
5	2. xi. 1931	3	26·59	0·727	107	40·62	1·015	420	29·22	0·893	106
20	8. ix. 1932	4	30·48	0·712	92	45·94	1·124	484	28·28	0·924	96
21	3. x. 1932	5	29·49	0·696	94	45·11	1·067	721	28·65	0·920	99
16	9. v. 1932	6	33·28	0·727	86	49·52	1·075	1231	34·30	1·027	88
18	17. vi. 1931	7	30·71	0·711	96	48·04	1·020	2064	31·00	1·039	95
23	14. xi. 1932	8	28·92	0·675	102	45·36	1·015	2314	30·57	0·926	106
		Average	29·71	0·709					30·10		

* On the end of this hour.

glycogen formation. They have been observed by many authors [Bornstein & Holm, 1928; Lusk, 1931]. The most pronounced discordance between the S.D.A. of glucose and glucose oxidation was obtained by Dann & Chambers [1930] in fasting dogs. Ingested glucose exerted a S.D.A. with almost complete absence of glucose oxidation and when even after fasting, glucose oxidation progressively improved, and the S.D.A. slightly increased, the latter factor was not at all proportional to the former. Although these dissociations, when slight, may be explained even on the basis of glucose oxidation [Lundsgaard, 1931; Grafe, 1934], it is impossible to do so on this basis when they are so great as in Chambers's experiments, and when they appear at the fat level of non-protein R.Q.

It has been shown in the author's former work [Table VII, 1931–5] that when 1 mol. of sugar is burnt, some 220 cal. of the S.D.A. are set free, i.e. one-third of the molecular heat content. This has been established only for one rate of continuous intravenous hexose supply, i.e. for 2 g./kg./hr. When the speed of sugar inflow was raised in the present work this relationship changed, and the supposed regularity disappeared. In spite of the fact that the rise of O_2 consumption by tissue slices when glucose or fructose is added is different in various

organs [Kisch, 1933], the effect on the whole organism seems to be similar for these two sugars, whether given by mouth [Carpenter & Fox, 1930] or by vein (Table VII).

(iv) *S.D.A. and storage of glucose in non-glucose form*

In this section that part of the glucose assimilated will be treated which remains after subtraction of the glucose oxidized, of the glucose transformed manifestly into fat and of that split into lactic acid and

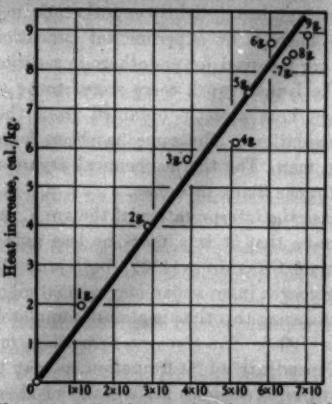

Fig. 7. Parallelism between the heat increase and the heat of glucose assimilated, non-oxidized, during the time S.D.A. lasted. Values from Fig. 2. At each point glucose infusion rate shown.

eliminated. This remainder is turned chiefly into glycogen. Here are also included all the reactions which have not been examined in this work and which are all summarized as "storage reactions" (Fig. 11).

As Fig. 7 shows, there is a parallelism between the excess heat and the heat of glucose assimilated but not oxidized (practically, glucose stored). A straight line may be drawn through the experimental points, passing through zero of the scale. Let us try again, as we did in part (iii) of this Discussion, to compare the deviation of heat increase referred to the metabolic channel in question with that referred to the total assimilation. Table VIII seems to suggest that the storage part of assimilation may well be the source of the S.D.A. rather than the oxidative one. The average heat increase as percentage of the heat of glucose stored,

amounts, for the entire period of the S.D.A., to 14·30 p.c. ± 1·04, minimum −2·24, maximum +2·89 p.c., and for the culminating part of the experiment to an average of 12·32 p.c. ± 1·17, minimum −2·34, maximum +2·92 p.c. The deviation of the percentage, though not so small as that found in reference to the total assimilation, is not far from it. This justifies the belief that it is among the storage reactions rather than among the oxidative reactions that the active parts of the mechanism of the S.D.A. should be looked for.

Glycogen formation, being slightly endothermic, needs a very minute fraction of heat. There is no experimental indication that important amounts of heat are removed for endothermic reactions during glucose transformation. In Lusk's work there seems to be satisfactory correspondence between heat directly obtained from the body and that calculated from oxidations. The same has been found by Burton & Murlin [1935] on man. The thermochemical arguments are, however, very insufficient for the living processes.

If we are to base the interpretation of the S.D.A. on glycogen formation, we must prove that it is a more or less independent reaction. According to Dann & Chambers [1932], both muscular and liver tissue are able to form glycogen in an apparently normal manner, although the oxidation of sugar during this time is almost completely suppressed and the S.D.A. persists [1930]. The glycogen formation, moreover, seems to proceed fairly independently of fat formation, as may be concluded from the work of Schur *et al.* on mice [1934], and of Henle & Szpingier [1936] on tissue slices.

It is possible that the rise of O_2 intake not accompanied by a corresponding rise of R.Q. at the beginning of glucose infusion is connected with the storage reactions occurring at this time. The contrary case is seen in Table X. In the seventh hour after glucose infusion the blood sugar was almost exactly at the same level as before the experiment. At this time the average heat production was only 1 p.c. higher than before the infusion. In the seventh hour after glucose infusion the non-protein R.Q. was quite close to the initial level of 0·709 only at the 1 g. rate of glucose supply. At all other rates it remained high above this level.

The same discrepancy is observed in Fig. 10. Between the 13th and 19th hour of experiment the CO_2 output remained 0·09 l./kg./hr. above the initial level. This equals 0·12 g. of glucose/kg./hr. which is burnt without rise of heat production. In the absence of the excess of glucose infused, the material burnt in all these cases without a rise of O_2 intake is supposed to be the excess glycogen formed from infused glucose.

(v) *Glycolysis and the S.D.A. of glucose*

After some products of glucose breakdown have escaped oxidation at the pyruvic acid stage, they may pass by oxido-reduction into lactic acid [Parnas, 1937] and so produce some heat. Lactic acid in the liquids of the body may be estimated at 0·15 g./kg. at the highest (Table V), and it undergoes constant dynamic changes characterized by its transformation into glycogen or some other kind of utilization in one group of organs, whereas the other group is constantly producing it from glucose.

It has been shown by the author [Table VII, 1931–5] and in collaboration with Sekuracki [1935] and Fiszel [1935, *a*, *b*] that there seems to be no parallelism between the S.D.A. and (1) the intensity, distribution and course of lactic acid diffusion from the organs into the blood, (2) the blood lactic acid level, and (3) the lactic acid elimination in the urine. The same S.D.A. is yielded by fructose transformation, in which the liver is the site of enormous lactic acid formation from fructose, other organs contributing only the fasting glycolytic quantities of lactic acid, as by glucose utilization, in which the usual glycolysis in various systems shows only a small increase [Table VII, 1934]. Strong fructolysis in a dog after 10 days' fast and moderate fructolysis after protein diet exhibit the same S.D.A. (Table VII, 1935).

In the present work the S.D.A. grows to some extent with the rising blood lactic acid level (Fig. 8), until at the 6 g. infusion rate there is a slight dissociation of both: the heat does not increase further, but lactic acid still does, until this increase also finally stops.

(vi) *Utilization of blood sugar by the organs, and the S.D.A.*

Although the appearance of the S.D.A. of sugar is connected with the appearance of the sugar introduced in the internal medium, their relationship is not so easy to detect. Certainly they do rise together to some extent (Fig. 9), but when the concentration of blood sugar passes the diabetic limit [Wierzuchowski, 1936, 1937] there is no further increase, whether of heat production or of oxidation and assimilation, although the blood sugar is being raised still further (Fig. 9). When, after each addition to the infusion rate of 1 g./kg./hr. the increments of the metabolic values are compared, an inverse relationship between blood-sugar rise and the increase of the metabolic values is detected: the higher the rise of blood sugar, the smaller the corresponding quota of glucose trans-

formed, until at a limiting rate of some 6 g. of glucose/kg./hr. the maximum rise of blood sugar is obtained with negligible or no rise of heat production, oxidation or assimilation (Fig. 9). Conceived in this incremental way, the inverse relationship of blood sugar increase and rise of assimilation discloses itself as being in connexion with the S.D.A.

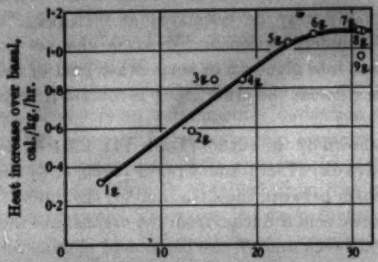

Fig. 8. Specific dynamic heat increase during the fourth to sixth hour of glucose supply plotted against the increase of blood lactic acid (from 6 infusion hours) over the preliminary value, in two dogs. From Table V. At each circle the glucose infusion rate is given.

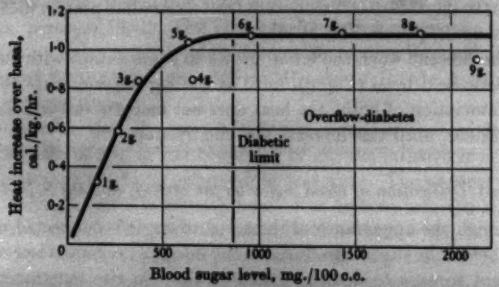

Fig. 9. Relationship of the S.D.A. to the corresponding average blood sugar level at the peak of glucose action (fourth to sixth infusion hour). At each circle is given the corresponding infusion rate. From Table V.

If the amount of glucose removed from the blood by the kidneys is subtracted from the whole glucose disappearing from the blood, the remainder represents the total assimilative removal and this value, according to our findings, must be closely proportional to the excess heat production. The same is the case with other hexoses, according to the author's investigations [1931, 1935].

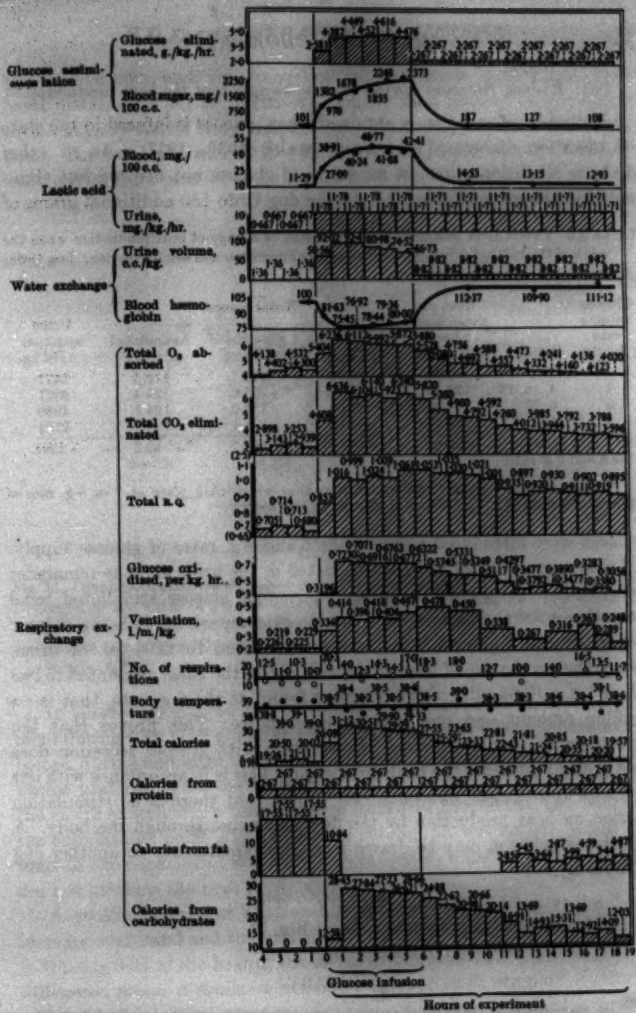

Fig. 10. During 6 hr. glucose was infused at the 9 g. rate per kg./hr. Dog Ordo. Exp. 19 (10. xi. 1934). Body weight 10·52 kg., urine N 0·101 g./hr., body surface (M.–R.) = 0·538 sq. m., (C. & D.) = 0·500 sq. m. Values represent the highest ability of the resting dog to dispose of glucose. In the fourth hour some derangement of kidney function sets in, characterized by the diminished flow of urine. This persists until the end of infusion.

(vii) *Kidney function during glucose supply and the heat production*

Valuable information is obtained when glucose is infused in the state of overflow diabetes [Wierzuchowski, 1936, 1937]. As in other diabetic conditions there is no S.D.A. of glucose not utilized but eliminated in the urine (Table XI). In the dog Ordo 180 additional grams of

TABLE XI. Twelve hour balances showing the lack of change of heat production when the work of the kidneys is being raised by sugar elimination and urine secretion. Dog Ordo; values calculated for 10 kg. dog.

Glucose infusion rate g./kg./hr.	Exp. no.	Heat production over basal cal./10 kg.	Total glucose: Infused g./10 kg.	Total glucose: Eliminated g./10 kg.	Total glucose: Assimilated g./10 kg.	Urine secretion c.c./10 kg.
6	3	73·72	360·0	243·7	116·3	2471
7	4, 16, 18	76·38	420·0	295·6	124·4	3927
8	5, 20	74·15	480·0	355·6	124·4	4989
9	19	74·36	540·0	406·4	133·6*	5762
Difference between the 6 and 9 g. rate		+ 0·64	+180·0	+162·7	+ 17·3	+2291

* There is some excess of utilization over that which took place at 7 or 8 g. rate of infusion, because of the retention of glucose by the kidney.

glucose were introduced between the 6 and 9 g. rates of glucose supply. Of that amount 162·7 g. were eliminated in the urine and the remainder was utilized (17·3 g.). This huge amount of glucose stimulated renal secretion so that it was almost double in comparison with the volume found at the 6 g. rate: the increase amounted to 2291 c.c. of urine. Nevertheless the rise of heat production above the basal amounted to less than 1 p.c. (0·64 cal.) of the quantity found at the 6 g. rate, that is—a trifling amount and one without significance. This proves that the great rise of kidney function in connexion with glucose secretion does not of itself influence the heat production. This is in accordance with our present knowledge. The same fact proves that there is no stimulating action on heat production by the flow of glucose through the body. A similar conclusion may be drawn from the experiments on another dog (Table X).

(viii) *Conclusion*

This is shown in graphical form in Fig. 11.

SUMMARY

In two female dogs the respiratory exchange was examined during long periods. The basal heat production showed a standard deviation of ± 4·3 p.c., the body weight ± 1·3 p.c., lung ventilation ± 7 p.c., surface

area ±0·68 p.c., and the frequency of respiration ±10·6 p.c. Their basal metabolism per sq. metre was higher than normal in association with a slightly raised body temperature, due to the experimental conditions. The total R.Q. was 0·722±0·013. About 12 p.c. of the basal heat was derived from protein and the rest from fat.

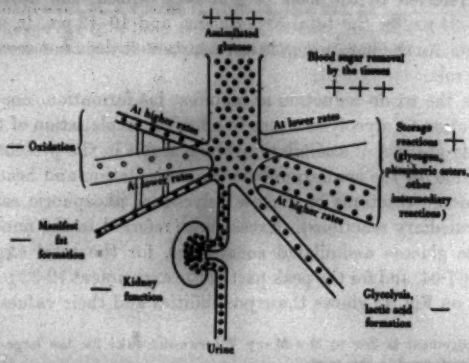

Fig. 11. Channels of removal of glucose molecules injected into the blood stream, and the origin of the S.D.A. of glucose according to present work. The thin line shows the percentage oxidized or stored at lower rates of infusion, while the thick line shows the same at higher rates. The probability of S.D.A. originating in the corresponding channel is indicated by + or −.

In these dogs glucose was infused at a constant rate in each experiment during 6 hr. (1–9 g./kg./hr.), and the respiratory exchange examined before, during and after infusion. As a result of the dosage of glucose the excess heat rose with the increasing rate of glucose supply, showing in the entire experiment a total increase from 1·97 to 8·68 cal./kg., while at the culminating point a rise of 0·3–1·1 cal./kg./hr. was noted. When the limit of the capacity of the body was exceeded, a further rise of glucose did not increase the heat production.

A strikingly regular relationship appears between the entire heat increase over basal and the total assimilation of glucose, the former being 9·30 p.c. ±0·34 of the heat of the latter within the whole range of glucose utilization rates. A similar connexion is discovered at the peak of glucose action, at all injection rates, namely, 8·74 p.c. ±0·61. It is therefore most probable that the specific dynamic action of glucose is proportional to the quantity of glucose assimilated, not only in the whole experiment, but

also in each fraction of it, each cal. of assimilated glucose liberating 0·093 cal. of excess heat. The same connexion must also be granted between the S.D.A. and the assimilative removal of blood sugar. The non-assimilated part of glucose is eliminated by the kidneys and does not play any part whatsoever in the rise of heat production.

When referred to the heat of glucose oxidized, the heat increase affords 17–34 p.c. in the total experiment, and 19–42 p.c. in its culminating part. As the percentage is not constant it does not seem to play a deciding role.

Neither the oxido-reduction as manifest fat formation, nor as lactic acid production by glycolysis seems to afford an explanation of the S.D.A.

The only channel of assimilation which seems in the present work to be responsible for the connexion between assimilation and heat increase are the storage reactions of glucose (glycogen, phosphoric esters, and other intermediary reactions). Excess heat referred to the non-oxidized part of the glucose assimilated constitutes, for the total experiment, 14·30 p.c. ± 1·04, and for the peak part of the experiment 12·32 p.c. ± 1·17. The graph on Fig. 11 shows these possibilities and their values.

Acknowledgement is due to Mrs Mary Wierzuchowski for the large number of calculations performed in this work.

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J. Physiol. (1937) 91, 172–185 612.733

THE RELATION BETWEEN THE MECHANICAL AND ELECTRICAL ACTIVITY OF A MOLLUSCAN UNSTRIATED MUSCLE

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In two other papers [Fletcher, 1937*a*, *b*] I have shown that the anterior retractor of the byssus of *Mytilus edulis* is an unstriated muscle whose constituent fibres are arranged in parallel to one another, and all extend from end to end of the muscle. This simple structure enables the action potential to be recorded without serious distortion. It is found to be closely similar to the action potential of other excitable tissues.

The present paper is concerned with the relation between this action potential and the mechanical activity of the muscle.

Apparatus

The procedure which was followed and the electrical recording devices which were used in these experiments were described in my first paper. The muscle was suspended vertically in a special moist chamber, and the action potentials were recorded by an oscillograph writing on smoked paper after they had been amplified by a four-stage directly coupled amplifier.

The myograph. The thread which was tied round the upper end of the muscle passed out of the top of the muscle-chamber and was attached to one end of an isotonic lever which was in turn connected by a further thread to a torsion wire myograph. This had a fine aluminium pointer arranged to write on the smoked paper immediately below the oscillograph pointer. Both levers were supported on a Palmer stand. The advantage of this arrangement was that the muscle could be kept stretched at a constant tension by means of a weight hung on the opposite arm of the isotonic lever, while adjustments could be made for changes

in the resting length of the muscle (which were often very considerable) by raising or lowering the whole system on the Palmer stand until the thread joining the two levers was just taut. The magnification of the movement of the end of the muscle obtained by the torsion wire myograph was 40 : 1. The greatest deflexion ever observed was 4 cm., thus the muscle never shortened by more than 1 mm. (i.e. 2–3 p.c. of its length).

Action potential and associated contraction

Fig. 1 shows simultaneous records of action potentials and tension development in response to a series of maximal condenser discharges at intervals of 2 sec. Each action potential is followed by a small increase in the tension of the muscle.

Fig. 1. Electrical and mechanical responses to a series of brief shocks. (Condenser discharges $RC = 30$ msec., 7·5 V.) Temp. 14° C. Upper tracing: oscillogram. Lower tracing: myogram (isometric).

Note. The arrangement of the leads on the muscle is shown in this and in the subsequent figures by an accompanying diagram. Cross-hatching indicates the site of injury (by crushing the muscle with heated forceps). All the kymograph records in this paper are reduced to half their original size.

The question to be decided is whether the action potential and the associated tension development are causally related to each other, or whether they are independent responses to the stimulus. That the tension development should be the cause of the action potential is most unlikely; for the action potential always precedes the associated contraction. Moreover, as will be shown presently, contraction may occur in the absence of any action potentials. On the other hand, the action potential might well be the direct effect of the stimulus and might then, in its turn, be stimulating the contractile mechanism; for this is generally supposed to be its function in striated muscle. If this hypothesis is correct, variation in the size of the action potential would be expected to result in parallel variation in the amount of associated tension development. This expectation can be put to the test in several ways.

(1) *Variation in the strength and polarity of the stimulus*

Observations were made of the size of the action potential and the tension produced with stimulating discharges of different strengths and polarities. In carrying out such an experiment allowance has to be made for certain peculiarities of the contractile response of this muscle.

(*a*) The amount of tension which is developed in association with a single action potential is extremely small. For instance, in Fig. 1, which is a typical case, it was about 0·7 g. and the stimulus was nearly maximal. The amount of tension produced by a single small discharge was almost beyond the sensitivity of the recording system employed. For this reason multiple stimuli were used.

(*b*) The muscle is very easily fatigued. For this reason, submaximal contractions were always observed, the number of discharges at each application being no more than was necessary to give an easily detectable contractile response.

(*c*) There is a very marked staircase effect in the contractile response of this muscle as Winton [1936] has shown. This staircase effect is to be carefully distinguished from that of the action potential itself [Fletcher, 1937*b*]. If a group of discharges is applied to the muscle at 2 sec. intervals, the resulting action potentials often show a staircase, the last of the group being larger than the first (this is only slightly evident in Fig. 1). On the other hand, if a series of such groups is applied to the muscle at intervals of about 2 min., the average size of the action potentials in each group will remain constant while the contraction associated with each group will steadily increase to reach a maximum. This fact is illustrated by Fig. 2 in which the average size of the action potentials of a series of groups of nearly maximal discharges and the tension associated with each group are plotted against time. When the groups are at intervals of 2 min. the contraction increases steadily to a maximum: when the interval is lengthened it decreases, while after a prolonged maximal tetanus it increases again. Meanwhile the action potentials remain almost unaltered in size. This independence of the staircase effect of the contraction from any corresponding changes in the action potential means that the relation of the size of the action potential to the associated tension development will only be significant when the contractile mechanism is maintained at a particular "level" on the staircase.

In Fig. 3, the average height of the action potentials due to groups of ten stimulating discharges, both anodal and kathodal,[1] of various

[1] A stimulus is called "anodal" when the stimulating lead nearer the recording leads was the anode (as in the diagram in Figs. 1 and 2) and "kathodal" when it was the kathode.

strengths, applied at intervals of 2 sec. and the amount of tension development associated with each group, have been plotted against the

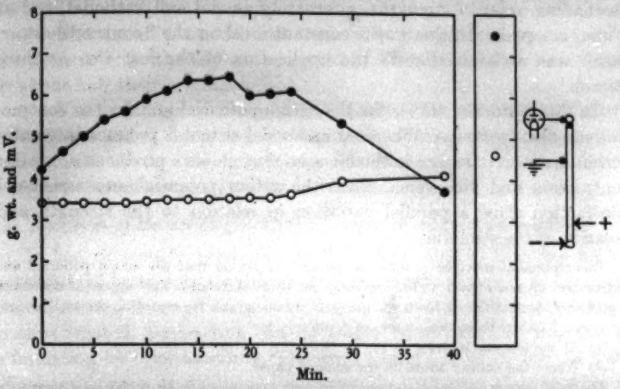

Fig. 2. The "staircase effect" of the contraction (see text). Hollow circles: average height of the action potentials in each group. (Stimulus: 20 condenser discharges $RC = 10$ msec., 10 V. at 1 per sec.) Solid circles: associated tension development (isometric). Temp. 13° C.

The points in the column on the right represent the values observed after a maximal tetanus of 2 min. duration which developed 40 g. tension.

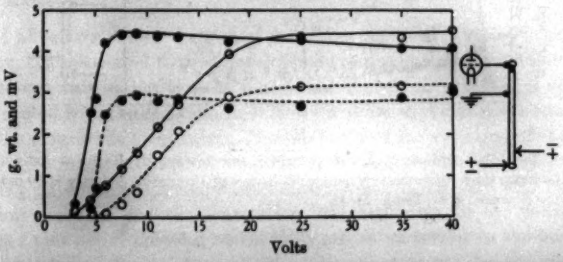

Fig. 3. Strength-response curve for action potential and for contraction (isometric). Ordinates: action potential and tension development. Abscissae: strength of stimulus. Continuous line: action potential. Dotted line: tension development. Solid circles: anodal stimuli. Hollow circles: kathodal stimuli. Stimuli: condenser discharges. $RC = 30$ msec. Temp. 14° C.

voltage of the stimulus. Before the experiment was started, a series of maximal groups of discharges was applied to the muscle at 2 min. intervals

until a constant mechanical response had been attained for three successive groups. The actual experimental stimuli were then applied in descending order of strengths, alternately anodal and kathodal, and at 2 min. intervals. In this way a constant level on the "contractile staircase" was maintained until the application of the last two or three stimuli.

In this figure the curves for the action potential and for the contraction are closely similar. Wherever an anodal stimulus produces a greater action potential than a kathodal stimulus, it also produces a greater contraction and vice versa. Thus the action potential and associated contraction show a parallel variation in relation to the strength and polarity of the stimulus.

This statement must be qualified at present by saying that the action potential and contraction do not appear to have exactly the same threshold. This apparent difference is probably attributable to the inadequacy of my myograph for recording the smaller contractions: for there were two sources of friction in it:

(*a*) At the isotonic lever.

(*b*) Where the pointer wrote on the smoked paper.

The ease with which such sources of friction may produce an apparent discrepancy between the thresholds of the mechanical and electrical responses of muscles is well known [Fulton, 1926, p. 227].

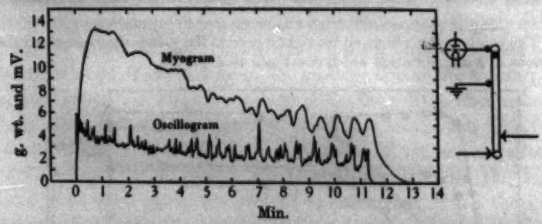

Fig. 4. See text. Ordinates: action potential and tension development (isometric). Abscissae: time. Stimulus: condenser discharges of alternating polarity at 30 per sec. *RC* = 30 msec., 9 V. Temp. 14° C.

(2) *Random variations in the size of the action potential*

When a series of brief currents is applied to the muscle at a frequency greater than 5 per sec., the resulting action potentials fuse together almost completely. The fused action potential may have a regular plateau owing to complete asynchronism in the responses of the individual fibres of the muscle, or it may have an irregular plateau owing to some degree of synchronism [Fletcher, 1937*b*]. An example of such an irregular fused response to a prolonged repetitive stimulus is given in Fig. 4 in

which the records of the action potential and of the associated contraction have been traced out, corrected to linear ordinates, and superimposed. Two points are to be observed:

(*a*) The action potential and contraction fatigue at approximately the same rate: at the end of the period of stimulation, both have fallen to about half their original height.

(*b*) The periodic variations in the tension of the muscle, particularly towards the end of the period of stimulation, correspond with the largest spikes in the electrical record. Whenever there is an increase in electrical activity, there is also an increase in tension.

(3) *Abolition of the action potential by means of magnesium solution*

It has previously been shown [Fletcher, 1937*b*] that the action potential can be completely abolished by soaking the muscle in an adequate concentration of magnesium chloride in sea water. When the muscle is thus treated, contraction can no longer be obtained in response to stimulation by brief currents. (The word "brief" must be used in spite of its ambiguity. It will be shown presently that even after soaking in magnesium solution the muscle will contract in response to a prolonged galvanic current; but the minimum duration of current which will result in detectable tension development after magnesium treatment has not been determined exactly. In fact the "brief" currents were condenser discharges of time constant (*RC*) of not more than 40 msec.)

I have now shown that when the action potential is varied in several ways, the associated tension development varies in a closely similar way. Although this makes it seem likely that the action potential is closely connected with the contraction, it does not prove that it acts as a stimulus to the contractile mechanism. The similarity of the variation of the two activities might be purely fortuitous, or it might be due to the stimulus acting on some preliminary mechanism which in its turn sets off the action potential and the contraction independently; for the variations in the two activities would then both reflect variations in the activity of this preliminary mechanism. More direct evidence as to the relation between action potential and contraction is to be found in a consideration of the electrical and mechanical responses of the muscle to galvanic stimulation.

The electric response to a galvanic current has been described in an earlier paper [Fletcher, 1937*b*]. It consists of a single action potential at the beginning of the stimulus, followed typically by no further

conducted action potentials but by local electrotonic potentials. If the stimulus is anodal, the only contraction is a small one associated with the initial action potential. If the stimulus is kathodal, however, the tension continues to increase throughout its application, until it reaches a maximum whose value depends upon the strength of the stimulus. These responses are illustrated in Fig. 5. In this case the small contraction associated with the initial anodal action potential is almost imperceptible, but there is a slight relaxation to be seen where the kymograph was stopped between the two records. Since there are no associated action potentials, this kathodal galvanic contraction must be the result of the

Fig. 5. Electrical and mechanical responses to galvanic current. A. Anodal stimulation. The oscillogram (upper tracing) shows a single action potential followed by anelectrotonus. The myogram (isometric) shows a very slight contraction. B. Kathodal stimulation. The oscillogram shows a single (smaller) action potential followed by katelectrotonus. The myogram shows a marked contraction which shows no sign of relaxation after 13 sec. (cf. Fig. 7A). Stimulus: 4 V. Temp. 17° C.

stimulus acting directly on the contractile mechanism of the muscle. That the single initial action potential is not responsible for the contraction produced by the kathodal stimulus may be shown by applying the galvanic stimulus to a muscle treated with magnesium, when the initial action potential is abolished but the contraction is still obtained.

To have shown that the contractile mechanism can be stimulated directly does not imply that it cannot also be stimulated by action potentials. Both methods of stimulation may be possible. If this were so, however, it would be surprising if the contractions resulting from the two kinds of stimulation were found to be identical. The direct effect of the stimulus should be localized to the immediate neighbourhood of the stimulating leads, while the effect of the action potentials should, by reason of their conduction away from the stimulating leads, be expected to be evenly distributed over the whole muscle. In order to see whether

or not the two kinds of contraction could be distinguished in this way, advantage was taken of the large amount of shortening of which the muscle is capable when it is allowed to contract isotonically. Photographs were taken of the muscle in maximal isotonic contractions induced by repetitive and galvanic stimuli. Examples of these photographs are given in Fig. 6.

For the taking of these photographs, the muscle was suspended in an open ebonite trough, with a centimetre scale behind it. A thread at one end was attached to the bottom of the trough, and one at the other end to an isotonic lever. Cooled sea water was dripped over the muscle continuously, except during actual moments of stimulation. Current for stimulation was led to it by two threads moistened with sea water.

Fig. 6. Photographs of a muscle suspended isotonically. A. Relaxed. B. Contracted in response to a tetanic stimulus. (Condenser discharges at 10 per sec.) C. Contracted in response to a galvanic stimulus. (Upper string kathode.) The scale behind the muscle is in centimetres.

The distinction between the two kinds of contraction is quite clear. In photograph A the fully relaxed muscle is shown. In B it is shown at the peak of a contraction induced by a repetitive stimulus: the whole muscle is evenly contracted. In C it is shown at the peak of a contraction in response to a galvanic stimulus: shortening and thickening occur most obviously in the immediate neighbourhood of the upper string electrode which was the kathode.

This experiment provides evidence that the action potential is normally responsible for the excitation of the contractile mechanism throughout the whole muscle, but that the mechanism can be locally excited by the direct effect of a sufficiently prolonged galvanic stimulus.

The "after potential"

After a response to a repetitive stimulus, the return of the oscillogram to its base-line was often noticeably delayed. A possible interpretation of this after potential is that it is a "contraction potential". Bishop & Gilson [1927], in their well-known investigation into the shape of the monophasic action potential of the sartorius muscle of the frog, showed that there was a long "tail" potential which bore a close relationship to the contractile response of the muscle, and they interpreted it as an electrical sign of the contraction. In the anterior retractor muscle the contraction associated with a single stimulus is extremely small, and it is not surprising to find little or no corresponding "tail" in a single action potential. On the other hand, after a repetitive response, with a large associated contraction, we might well expect to find one. This interpretation of the after potential finds support in the following facts:

(*a*) The after potential is always more prominent when the tension development is large.

(*b*) Most of the decline takes place during the relaxation of the contraction. This fact is illustrated in Fig. 7 A in which a base line has been drawn in to make the return of the oscillogram clearer.

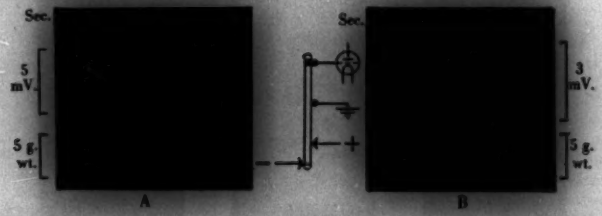

Fig. 7. The after potential. Upper tracings oscillogram: lower tracings isometric myogram
A. Stimulus: condenser discharges, $RC = 7$ msec., 14 V. at 7 per sec. Temp. 14° C.
B. Stimulus: condenser discharges, $RC = 10$ msec., 10 V. Single and 12 per sec. Temp. 15° C. The kymograph was stopped at the summit of the contraction after the end of the first part of the after potential, and was then moved on at intervals during the relaxation.

These two observations indicate that at least some of the after potential is attributable to the contraction of the muscle, but as Fig. 7 B shows, a considerable amount of decline of the potential may occur before the muscle has relaxed at all. That this part of the decline is not just the normal slow descending phase of the action potential may be seen by comparison with the preceding single action potential. This first part of the after potential may be compared with the "retention of negativity" observed by Levin [1927] in crab's nerve after a short tetanizing stimulus. Levin, however, found that the nerve was completely depolarized after 2 min. tetanization, and that it took 10 min. to recover. Fig. 4 shows that the action potential in the anterior retractor muscle is only about 50 p.c. reduced in size after 7 min. tetanization; moreover the first part of the after potential never occupied more than 5 or 6 sec.

The galvanic contraction and tonus

Local galvanic contractions like that illustrated in Fig. 6C have been described in many kinds of unstriated muscle [Grützner, 1904], but have usually been attributed to excitation of the short muscle fibres lying immediately underneath the stimulating leads. In the anterior retractor the fibres run the whole length of the muscle and the excitation must be limited to a part of each fibre. There is nothing very surprising in this, however, for it is well known that the long-fibred sartorious

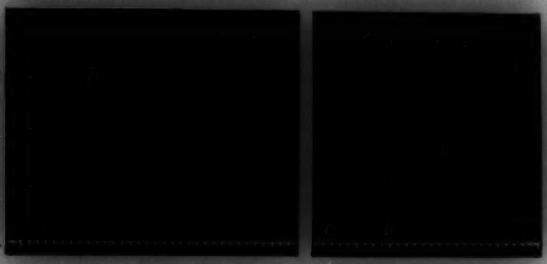

Fig. 8. Kymograph tracing of a muscle suspended as in Fig. 6. This muscle had undergone a tonic contraction during dissection. From *A* to *B* the slow relaxation of the muscle under a tension of 20 g. can be seen. At *B* a repetitive stimulus was applied for 5 sec.: after a small contraction there is a rapid relaxation. Between the two tracings there was an interval of 5 min. At *C* a repetitive stimulus was again applied for 15 sec.: there is a strong contraction which relaxes almost immediately. At *D* a galvanic stimulus was applied for 25 sec.: the contraction long outlasts the stimulus. At *E* another repetitive stimulus of 5 sec. was applied: the galvanic contraction is terminated like the original "tonic" contraction.

muscle of the frog may show a small, local, kathodal contraction during the passage of a constant current. Now in the anterior retractor, the types of contraction associated with excitation by a galvanic current and by repetitive discharges differ not only in the extent of their propagation, but also in their speed of relaxation, as Winton [1937] has shown. The "tetanic" contraction (i.e. induced by repetitive discharges) relaxes almost immediately, while the galvanic or "tonic" contraction is associated with a large increase in the viscosity of the muscle and is maintained long after the end of the stimulus. The galvanic contraction is further characterized by the fact that it may be abruptly terminated by the application to the muscle of a tetanic stimulus of sufficient strength and duration (Fig. 8).

During dissection, when the base of the byssus was being divided in the midline, the muscle often contracted suddenly [Fletcher, 1937*a*]. Since this contraction involved the whole muscle evenly and occurred when the base of the byssus and not the muscle itself was being cut, it was probably not attributable to local damage to the muscle, but possibly to excitation of nervous origin. Such a contraction was of a "tonic" type: that is to say, it persisted indefinitely if the muscle was left alone, and no electrical activity was ever detected in association with it. If, however, a brief tetanic stimulus was applied to a muscle which was maintaining such a contraction, relaxation occurred immediately (Fig. 8).

This "natural" tonic contraction is thus similar to the galvanic contraction in that it is maintained without appreciable action potentials and is terminated by the application of a tetanic stimulus. These facts indicate that the galvanic contraction is probably of physiological and not merely physico-chemical interest.

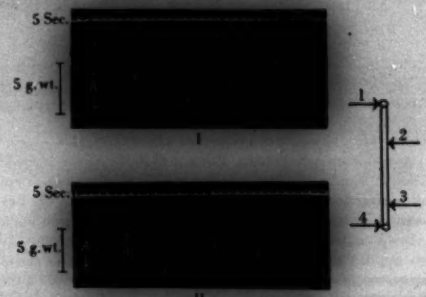

Fig. 9. The reversal of a galvanic contraction by a tetanic stimulus applied locally and at a distance, before and after treatment with magnesium. I. Muscle soaked in sea water. II. Same muscle after 15 min. in Mg solution (30 p.c. isotonic $MgCl_2$ in sea water). At *A* a galvanic stimulus of 4 V. with lead 1 anode and lead 2 kathode was applied: a galvanic contraction occurs. 15 sec. later a tetanic stimulus (alternating condenser discharges $RC = 20$ msec., 10 V.) was applied for 5 sec. through leads 3 and 4: after a latent period of about 5 sec. partial relaxation occurs. At *C* the same stimulus was applied through leads 1 and 2: the relaxation is completed. Stimuli A and C were then repeated to show the complete relaxation obtained by stimulus C alone. The arrows in each case mark the onset of the period of stimulation. Temp. 16° C.

The mechanism of the reversal of a tonic contraction by a tetanus

A few experiments were devoted to this problem. Their results are not yet clear enough to form a basis for discussion, but are worth describing briefly. The facts that have at present been determined are the following:

(*a*) The reversal of a tonic contraction is unaffected by concentrations of magnesium

which abolish the action potential, but it is itself abolished by higher concentrations (over 50 p.c. isotonic MgCl in sea water). This shows that the reversal is not caused by the action potentials nor by the contraction associated with the tetanic stimulus (cf. Fig. 9).

(*b*) A tonic contraction imposed at one end of a muscle by a local galvanic stimulus may be partially reversed by a tetanic stimulus applied at the other end of the muscle. It may, however, be more effectively reversed if the tetanic stimulus is applied at the same end. This reversal at a distance is unaffected by concentrations of magnesium sufficient to abolish conducted action potentials. Thus the reversal effect appears to be conducted incompletely from one end of the muscle to the other independently of conducted action potentials (Fig. 9).

(*c*) There is a latency which may amount to as much as 5 sec. between the end of the tetanic stimulus and the onset of the reversal. This latency is normally hidden by the contraction which is also produced by the tetanic stimulus, but it is easily seen in a muscle which has been treated by magnesium (Fig. 9).

Discussion

The idea that there are two mechanisms involved in the process of excitation of a striated muscle has been well known, and generally accepted, since it was first proposed by Bernstein in 1871. The stimulus, whether direct or indirect, sets off a propagated disturbance (Lucas) which is conducted over the whole muscle-fibre, is associated with the action potential, and sets the contractile mechanism into activity in its wake.

In the present paper reasons have been given for supposing that the same chain of events occurs when the anterior retractor is acted upon by a brief stimulating discharge. The evidence may be summarized as follows:

(*a*) The action potential always precedes the contraction.

(*b*) When the size of the action potential is varied in any way, the amount of associated tension development varies in a closely similar way.

(*c*) The whole muscle contracts in response to a local stimulus when and only when conducted action potentials are also developed.

The relationship, therefore, between the mechanical and electrical activities in the anterior retractor is much the same as in striated muscle. There are, however, some differences:

(1) In the anterior retractor the staircase effect of successive contractions associated with identical electrical responses shows that the relationship of the two mechanisms is not so rigid here as in striated muscle. An intermediate type is found in cardiac muscle: for Adrian [1921] has shown that if the interval between successive stimuli to the frog's heart is altered the contractions are of different heights, but the associated electrical changes are identical.

(2) In response to a single discharge, the anterior retractor develops only a very small fraction of the maximum tension of which it is capable. A muscle in good condition is usually capable of producing 50 g. tension

in response to a prolonged maximal tetanus, but a single action potential is seldom associated with the development of more than 1 g. tension. In striated muscle, on the other hand, a single twitch develops about one-fifth to one-half of the tension of a maximal tetanus.

(3) Direct excitation of the contractile mechanism is easily obtained in the anterior retractor by means of a galvanic stimulus. Direct excitation can be achieved in striated muscle by the use of minutely localized stimulation [Gelfan, 1930] and may occur in some muscles with galvanic stimulation [Bremer, 1932], but it is not so easily or regularly obtainable as it is in the anterior retractor muscle. In the latter, moreover, there is reason for supposing that the galvanic contraction is of physiological interest, on the grounds of its analogy to a supposedly natural tonic contraction of the muscle. If this argument, which is based on analogy, is acceptable, an interesting contrast may be made between the mechanisms adopted by striated and unstriated muscles for the development of tonic and phasic contractions. In both sorts of muscle quick, phasic contractions are the result of conducted action potentials. In striated muscles the tonic contraction is of the same type and is maintained by asynchronous, alternating contractions of the constituent fibres of the muscle, the amount of tone being determined by the number of fibres which are each contracting in an all-or-nothing fashion under the influence of conducted action potentials. In unstriated muscle, on the other hand, it appears that tonic contractions may occur without the mediation of conducted action potentials, and may thus be localized to a part of each fibre. The tone of the muscle as a whole would thus be determined by the amount of each fibre that is contracted and not solely by the number of contracted fibres.

Further evidence for the existence of such local contractions is to be found in a consideration of the histology of the anterior retractor muscle. In a transverse section of a supposedly, but probably incompletely, relaxed muscle, groups of contracted fibres are often seen [Fletcher, 1937*a*]. If serial sections are cut, there may be large fibres at one level in the right half of a section and none on the left, while a little further on, in another section, the contracted fibres will only be found on the left and not on the right, and so on. Thus the contracted fibres do not appear to run the whole length of the muscle. Since all the fibres have been shown by electrical means to run the whole length, these "contracted fibres" must just be the contracted parts of fibres, the remaining parts of which are relaxed.

Bozler [1927, 1928, 1931] has shown that the contractions of single unstriated muscle fibres obey an all-or-nothing law. My experiments make it seem likely that this all-or-nothing behaviour is imposed upon them, as it is imposed upon striated muscle fibres [Adrian, 1922], by an all-or-nothing action potential which is responsible for the onset of

the contraction. It is possible that the anterior retractor muscle is unique among unstriated muscles in its possession of this simple and characteristic action potential. It seems more likely, however, that its uniqueness lies rather in its extremely simple structure which allows its simple action potential to be recorded without gross distortion, and that a conducted action potential is a normal and essential part at least of the phasic contractions of unstriated muscles.

Summary

1. In the anterior retractor of the byssus of *Mytilus edulis* the action potential is followed by contraction of the muscle.

2. When the size of the action potential varies either spontaneously or as the result of variations in the stimulus, the associated contraction varies in a parallel way: when the action potential is abolished by the application of magnesium to the muscle, no contraction can be obtained in response to stimulation by brief currents.

3. When a prolonged galvanic current is applied to the muscle it will contract in the absence of conducted action potentials. This contraction is localized to the neighbourhood of the kathode, while the contraction in response to a tetanic stimulus, with its associated action potentials, is distributed over the whole muscle.

4. The galvanic contraction is analogous to a tonic contraction which was occasionally developed in the dissection of the muscle.

5. It is concluded that in the phasic contraction of unstriated muscles the action potential plays the same essential part as it plays in the contraction of striated muscle but that tonic contractions may occur in the absence of conducted action potentials.

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THE CEREBRO-SPINAL FLUID PRESSURE OF MAN IN THE ERECT POSTURE

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In a subject with a small opening in the skull following a decompression operation it was observed that when the subject was standing or seated the taking of a deep breath was followed towards the end of the expiratory phase by a rise and then a fall of intracranial pressure. This observation appeared to be worthy of further investigation. It is the object of this paper to present the methods adopted and to discuss the results obtained.

Methods

In all, thirty-five subjects have been investigated. None suffered from any lesion causing increased intracranial pressure. While the subject sat in a chair with the legs immersed and moving gently in a tank of water at 44–45° C., plethysmographic records were taken of one digit of a hand by a method previously described [Bolton *et al.* 1936]. In three subjects the opportunity was taken to obtain records of ventricular fluid pressure. They had been admitted for ventriculography, and subsequent X-ray examination demonstrated a normal ventricular system. Immediately before the injection of air records of changes in ventricular pressure were obtained. A hollow needle of gauge 18 was inserted into the lateral cerebral ventricle in the region of the vestibule through a small trephine hole of not more than 1·0 cm. diameter, made several days previously. Without the loss of cerebro-spinal fluid the needle was attached by a closed-air system to a capsule which carried a mirror reflecting a beam of light on the moving strip of bromide paper. It was determined by experiment that to cause a beam of light to cross from one side of the paper to the other (6·5 cm.) the addition of 0·10–0·04 c.c. of fluid to the closed-air system was required, dependent upon the elasticity of the tambour. It

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was considered, therefore, that this arrangement permitted the recording of changes in pressure by a non-displacement or isometric method, for a change in volume of less than 0·10 c.c. in a total volume of *circa* 150 c.c. is negligible. In fifteen other subjects records of changes in the cerebro-spinal fluid pressure were taken by the insertion of a needle of gauge 18 into the cisterna magna. These subjects were to have the cisternal puncture in order that "lipiodol" might be injected for diagnosing the presence or absence of obstruction in the vertebral canal. Opportunity was taken of recording the cerebro-spinal fluid pressure when the puncture was made, and before the "lipiodol" was injected. Subsequent X-ray examination revealed no spinal block in these subjects. In twelve subjects lying on the right side records were obtained of pressure changes in the lumbar sac by a similar method. These records were taken during routine lumbar puncture. In several of the investigations two tambours of different elasticity were connected to the one needle. This was done to ensure that the complete sequence of changes would be available on the recording paper.

Arterial pressure readings were taken by the auscultatory method from one arm, the time of each reading being marked by a signal on the moving strip of bromide paper. Later these readings were transferred to the paper. Changes in arterial pressure were also recorded by the following method: Around the calf of one leg was placed a sphygmomanometer cuff, which was blown up to a few mm. below systolic pressure. A capsule placed over the dorsalis pedis artery of the same leg registered alterations in the size of the pulse wave. A larger pulse wave indicated a rise in blood pressure and a diminution or absence a fall in blood pressure.

Venous pressure was recorded from the jugular bulb. A hollow needle of gauge 16 attached to a syringe was inserted below the mastoid process and directed slightly forward and upward. At a depth of 3–5 cm. the vein was punctured, as indicated by the syringe filling easily with blood when a slight negative pressure was applied by withdrawing the plunger. The syringe was detached from the needle. The needle was then connected by pressure tubing to a reservoir containing 1 p.c. sodium citrate in normal saline, which was used periodically to flush through the needle and thereby keep its lumen patent. Inserted in the rubber tubing was a T-piece of 3 mm. bore, the upright of which acted as a manometer filled with the citrate solution. On clamping off the reservoir actual pressure readings could thus be obtained. Compression of the jugular vein below caused a rise in pressure, which fell immediately the compression was removed. This indicated that the needle was in the lumen of the jugular

bulb. To record the changes in pressure the top of the manometer was connected by a closed-air system to a suitable capsule with an attached mirror. During the taking of a record the reservoir remained clamped off. The point of the needle was directed in the jugular bulb very slightly in the direction of the cranial cavity. The subjects from whom venous pressure records were obtained were either seated or lay horizontal on a couch. In all experiments a record of the phases of respiration was taken by attaching a balloon to the chest wall with an elastic bandage. The balloon was connected to a similar capsule by a closed-air system.

Reference will be made to observations carried out on a subject before and after bilateral removal of the stellate ganglia and to other observations made during an operation for the removal of the stellate ganglion.

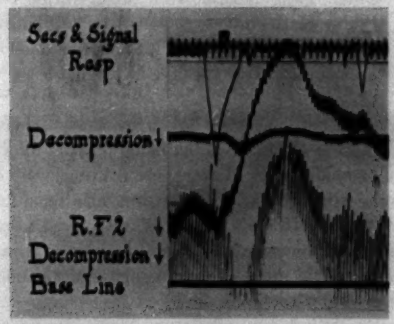

Fig. 1. Decompression. Sitting erect. Single deep breath. Two tambours of different elasticity are attached to a balloon over the decompression opening. Arrow after record from each tambour indicates direction of increase of cranial contents. The fainter tracing showing large oscillations with each pulse wave on the lower half of record is that from the lighter tambour. In this and succeeding figures in the record of respiration, the inspiratory phase is downwards. R.F. 2 in this and succeeding figures is volume record of right index finger. The arrow after points in direction of increase in volume. Line marked "Base Line" in this and following records is obtained from a mirror attached to a stand holding tambours. It permitted accurate measurements of pressure to be made, as without it the slight side-to-side movements of the paper over the roller could not be controlled.

Results

From the subject with the cranial decompression who provided the material for the original observation, records were obtained of the changes in volume of one finger and of the changes in intracranial pressure from the decompression opening by means of a balloon placed over the

opening and attached to the forehead with adhesive strapping. The subject was seated during the investigation. On taking a deep breath it was found that towards the end of the expiratory phase the decompression opening began to bulge, continued to do so for some seconds and then began to sink in, eventually to return to its normal position (Fig. 1). The plethysmographic records of the finger showed the usual marked diminution in volume commencing in the expiratory phase [Bolton, *et al.* 1936].

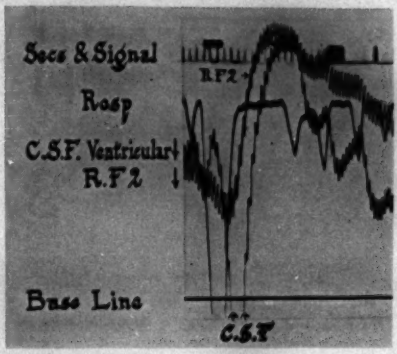

Fig. 2. Ventricular pressure. Sitting erect. Deep breath. Arrow following "Ventricular" is in direction of increase of pressure. At the signal the subject was requested to take a single deep breath.

Ventricular pressure

In three subjects, while in a sitting position, it was possible to obtain the record of changes in ventricular pressure accompanying the taking of a deep breath 15 times. On each occasion the direction of the changes was similar, though varying in amplitude. During the inspiratory phase a small fall in pressure occurred shortly after the commencement, but was rapidly replaced by a rise in pressure which continued into the expiratory phase, when it was replaced by a marked fall in pressure (Fig. 2). As a rule the fall in pressure was greater than the rise and always continued for a considerable time after the expiratory phase into the succeeding apnœic period. Thereafter the pressure gradually returned to the preceding level. The plethysmographic records of the digit showed changes as before. On quiet respiration there were slight fluctuations in intracranial pressure, a fall of pressure with inspiration and a rise with expiration.

It is to be observed that on taking a deep breath the predominant changes were a rise in ventricular pressure and then a profound fall during and after the expiratory phase.

Cisternal pressure

In eight subjects while seated records of changes in cisternal pressure were obtained 31 times in association with the taking of a deep breath. The cranial vertebral box was intact in these cases. The records were

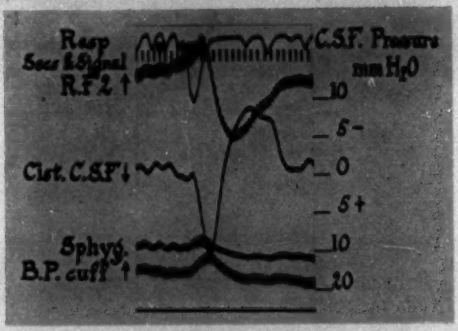

Fig. 3. Cisternal and blood pressure. Sitting erect. Deep breath. "Sphyg." refers to record from capsule on dorsalis pedis artery. "B.P. cuff" refers to alteration in pressure within cuff around calf. Other records as before. At series of signals subject was requested to take a deep breath.

similar in every way to those obtained from the ventricle and require no explanation (see Fig. 3). The changes during quiet respiration were of a similar character to those found in the ventricle.

Lumbar pressure

In twelve subjects while lying horizontal on the left side records were taken from a needle placed in the lumbar sac. On taking a deep breath there was a considerable fall in lumbar fluid pressure during the inspiratory phase. During expiration there was a rise of pressure of small amount and of short duration, which was followed by a more prolonged and profound fall in pressure for some seconds afterwards. The pressure then gradually returned to the normal level (Fig. 4). The changes in finger volume were no different from those described before. A study of the records during quiet respiration showed that there was no constant

relationship between the phases of respiration and the changes in lumbar cerebro-spinal fluid pressure.

It is therefore apparent that in the erect sitting posture the taking of a deep breath is associated with a rise and then a fall of pressure within the ventricle and the cistern. In the lumbar sac in the horizontal posture, there is first a fall and then a rise followed by a marked fall after the expiratory phase.

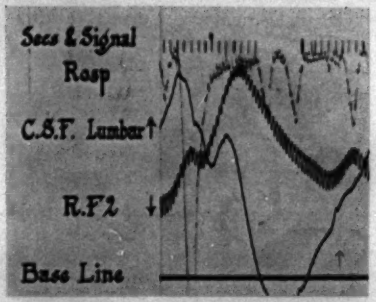

Fig. 4. Lumbar pressure. Horizontal posture. Deep breath. Arrow after C.S.F. indicates direction of rise in pressure. A few seconds previous to signal subject was requested to take a single deep breath.

Spinal block

In order to determine the alteration of pressure in the isolated lumbar sac and in the cistern simultaneously, subjects with a complete block of the spinal fluid system at a high level were investigated. In two subjects, each with a spinal tumour in the upper cervical region, simultaneous records of changes of cisternal and lumbar fluid pressure were taken when the subject was seated and was horizontal.

In subject A, while seated, compression of the jugular veins caused an increase of cisternal fluid pressure, but no alteration in lumbar fluid pressure. On the other hand, while lying horizontal, abdominal pressure resulted in a rise in lumbar fluid pressure only. These observations showed that there was a complete spinal block, the level of which was indicated by a "lipiodol" injection into the cisterna magna and a subsequent X-ray examination. When seated, on taking a deep breath, there was a rise in cisternal pressure followed by a fall after the expiratory phase, these changes being identical with those observed in the "normal" subject. In

the lumbar sac there was a fall of pressure coincident with the inspiratory phase, followed by a rise during the expiratory phase (Fig. 5). It will be seen that the changes in the cistern are similar to those found in a normal. On the other hand, the changes in the lumbar sac are quite different from those registered at the level of the cistern in a normal subject. Further, during quiet breathing in the cisternal region the changes in pressure were in phase with respiration, but in the lumbar sac the pressure changes were out of phase with respiration. On rotating the table so that the subject was now lying on his right side, a deep breath was associated with

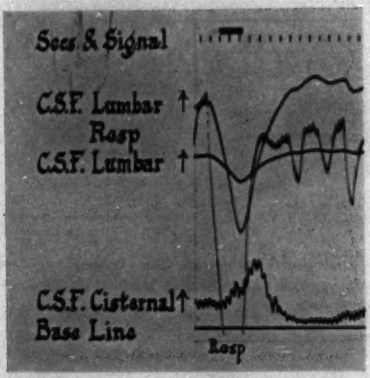

Fig. 5. Spinal block. Sitting erect. Deep breath. Two tambours of different elasticity were attached to needle in lumbar sac and one tambour to needle in cisterna magna. Arrows point in direction of increase of pressure. At signal subject was requested to take a single deep breath.

a fall in pressure at both the cisternal and lumbar levels during the inspiratory phase. This fall of pressure in the lumbar sac was replaced by a rise during the expiratory phase. On the other hand, in the cistern the fall was succeeded by a rise during the expiratory phase and then by a profound fall in pressure. With quiet breathing the phase of inspiration was associated with a fall in pressure and the phase of expiration with a rise in pressure at both sites.

In subject B, with a block at the same level, similar records were obtained, the changes in pressure being in the same direction as obtained in subject A, but of less magnitude.

Venous pressure

In two subjects in a sitting position, with a needle in the jugular bulb, records were taken of the changes in venous pressure in association

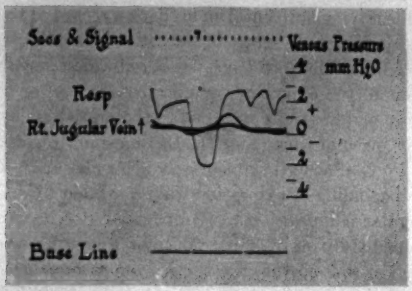

Fig. 6. Jugular pressure. Sitting erect. Deep breath. Two tambours of different elasticity were attached to the manometer recording jugular pressure. The tracings from each overlap. The pressure scale refers only to the tracing showing most fluctuation. Arrow is in direction of increase of pressure. At signal subject was requested to take a single deep breath.

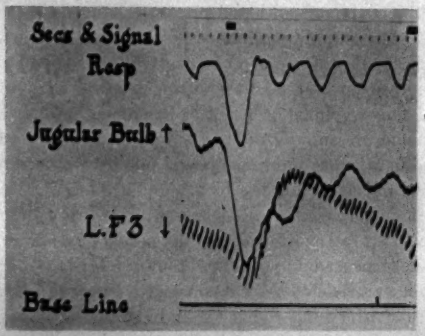

Fig. 7. Jugular pressure. Horizontal posture. Deep breath. One tambour attached to needle in jugular bulb. Arrow is in direction of increase of pressure. L.F. 3 refers to volume record of left middle finger. At signal subject was requested to take a single deep breath.

with a deep inspiration. The inspiratory phase was unaccompanied by any fall or rise in venous pressure, but during the expiratory phase a small rise of less than 1 mm. H_2O in venous pressure occurred with a subsequent

return to the preceding level (Fig. 6). No alterations of venous pressure were observed with the phases of quiet respiration. Jugular compression below the insertion of the needle caused a rapid rise in venous pressure, which returned to the normal base-line on release of the compression.

In one subject lying horizontal on his back a record of venous-pressure changes in the jugular bulb showed that with the inspiratory phase there was a considerable fall in venous pressure, which terminated at the commencement of the expiratory phase. At the end of expiration the venous pressure had not quite returned to the preceding level, but did so within a short space of time (Fig. 7). During quiet respiration a fall in venous pressure commenced immediately after the start of inspiration, and after the beginning of expiration was succeeded by a rise in venous pressure. Jugular compression caused a rapid rise in venous pressure which fell immediately on releasing the jugular compression.

In the erect sitting posture the rise in venous pressure occurred at a slightly later period than the rise in cisternal or ventricular pressure. There was no fall in venous pressure similar to the fall in fluid pressure.

Arterial blood pressure

In five subjects records of blood-pressure changes either by auscultation methods or by graphic qualitative methods were obtained, along with changes in cisternal pressure and of finger volume.

On taking a deep breath in the sitting posture changes of cisternal pressure and finger volume occurred as explained before. By observing changes in blood pressure by the method of auscultation during a series of deep breaths taken at intervals, it was possible to determine that towards the end of inspiration the blood pressure began to rise and then during the expiratory phase to fall, continuing to do so until below the original level. This fall continued into the period of respiratory rest. The blood pressure then gradually returned to normal (Fig. 8). In the records obtained from a capsule on the dorsalis pedis artery the size of the pulse wave from that artery was found to diminish at the commencement of inspiration, then to increase towards the end of the inspiratory phase and slightly before the rise in cisternal pressure. The pulse waves then commenced to decrease in size towards the end of expiration even to the point of disappearance, and only returned to their original size during or following the apnœic period. This latter diminution in size of pulse wave preceded by one or two beats the fall in cisternal pressure (Fig. 3). If the subject took a less rapid deep inspiration, the increase in cerebro-

spinal fluid pressure commenced during the inspiratory phase and coincided in direction and time with the changes in arterial blood pressure. On quiet respiration the changes in fluid pressure coincided with the changes in blood pressure.

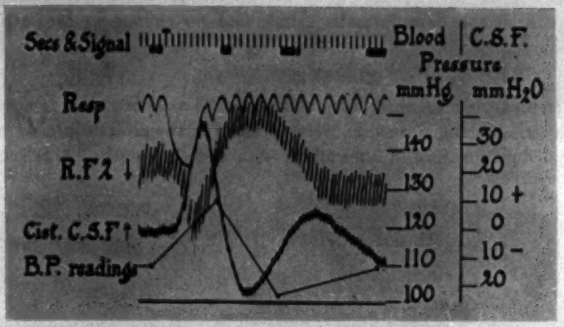

Fig. 8. Cisternal and blood pressures. Sitting erect. Deep breath. B.P. readings refer to figures obtained using a mercury manometer and the auscultatory method. At the upper signal subject was requested to take a single deep breath.

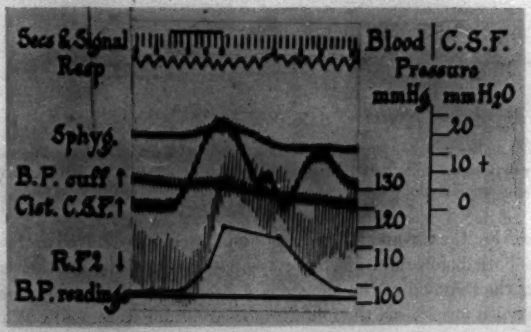

Fig. 9. Arterial and blood pressures. Sitting erect. Mathematical exercise. At long signal subject was requested to do some simple multiplication.

Several subjects, while seated, were requested to perform some simple mental arithmetic. This caused a diminution in volume of the finger, a rise in cisternal pressure and an increase in size of pulse wave from the

dorsalis pedis artery. The cisternal pressure changes thus coincided in time and in direction to the changes in size of pulse wave (Fig. 9). In the same subjects the light application of a small piece of ice to the skin of one or other forearm, trunk or thighs caused a diminution in finger volume of the same extent, but no alteration in the amplitude of the pulse wave from the dorsalis pedis artery and no change in the cisternal pressure.

Sympathectomy

In one subject several days before and 33 days after the removal of both stellate ganglia, records were obtained of the alteration of cerebro-spinal fluid pressure in association with a deep breath. These records

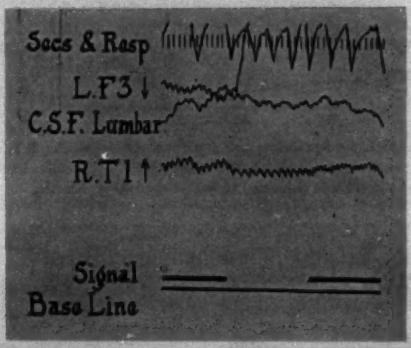

Fig. 10. Lumbar pressure. Horizontal posture. Stimulation of left stellate ganglion, during break in signal line. R.T. 1 refers to right large toe. This is a tracing taken from a photographic record.

showed that the rise and fall in intracranial pressure occurred as described above both before and after the sympathectomy. In another subject during the removal of the left stellate ganglion under intratracheal gas and oxygen anæsthesia the following observations were made. When the ganglion was stimulated by an induced electrical current after it had been separated from the thoracic ganglia and its preganglionic connexions severed, no change took place in lumbar pressure as the subject lay on his right side. Immediately following the stimulation diminution in the volume of the left middle finger occurred without any similar alteration in the volume of the right large toe (Fig. 10).

Discussion

It has been shown that in the erect sitting posture of man the changes in the cerebro-spinal fluid pressure in association with the taking of a

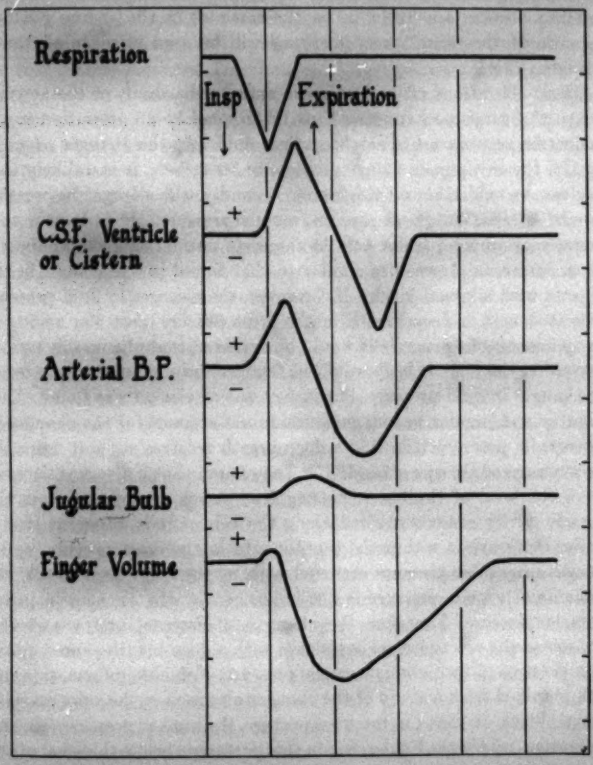

Fig. 11. Erect posture. Scheme of simultaneous changes in ventricular and cisternal pressure, arterial blood pressure, jugular venous pressure and finger volume on taking a deep breath.

deep breath, are a rise followed by a fall. The relationship between these changes and those occurring in arterial blood pressure, jugular venous pressure and finger volume is shown schematically in Fig. 11. The rise in

fluid pressure may occur before the end of inspiration provided the inspiratory phase is slow. In the erect sitting posture and erect standing posture, the cranio-vertebral system is in the same relationship to the thorax. It is therefore fair to assume that the changes found in the standing posture are likely to be the same as in the sitting posture. Accordingly the term "erect posture" will be used in place of "erect sitting posture".

These alterations of fluid pressure may be the result of the activity of one of several mechanisms. First, there may be an alteration in the production or removal of cerebro-spinal fluid. As the changes occur so rapidly, the two phases being complete in 30–40 sec., it is unlikely that they can be explicable on this basis. Secondly, the changes in pressure may be a reflexion of changes in venous pressure. It is known that compression of the jugular vein in the erect normal subject produces a rise in intracranial pressure similar to that found in the cistern in the subjects with a spinal block. If, however, the changes in fluid pressure associated with a deep breath in the erect posture were the results of changes in venous pressure it would be expected that changes in venous pressure at the jugular bulb would be found to be similar in character to the changes in fluid pressure, but in fact no correlation was found. That alterations in jugular venous pressure do not account for the changes in intracranial pressure following a deep breath receives support from the observations of Doupe *et al.* [1937]. They found that if a vein is situated above the level of the heart, no negative waves of pressure from the thoracic cavity are transmitted along the veins. It is apparent that a similar state exists within the jugular vein in the erect posture, as no changes in jugular pressure occurred with respiration. As a result, the intracranial venous pressure is not influenced by the changes in intrathoracic pressure. Therefore the changes in cisternal and ventricular pressure in the erect posture associated with a deep breath cannot result from changes in intracranial venous pressure. Valuable information has been obtained from a study of the changes observed in the subjects with a spinal block. In these, in the erect posture, the lumbar pressure fell with inspiration. Since the lumbar sac in this posture is below the level of the heart, the fall in lumbar pressure is most easily explicable as the result of a fall in venous pressure. At the same time the changes above the level of the clock are similar to those found in a normal subject and, as has been shown, do not result from changes in intracranial venous pressure. Thus in the normal sitting subject the changes of cerebro-spinal fluid pressure either in the cistern or ventricle were similar to those found

above the level of a high spinal block. Further, the changes in the isolated lumbar sac were different from those obtained above the level of the block or in a normal subject. Therefore the changes in a normal subject are not the direct result of changes occurring in the lumbar sac. From this it may be deduced that the changes in fluid pressure in the cistern or ventricle of a normal subject are not the consequence of alterations in general venous pressure. The changes of fluid pressure which take place in the isolated lumbar sac and which are the reflexion of the changes in venous pressure, become masked by changes in intracranial pressure brought about by another mechanism than venous-pressure changes, if the lumbar sac is in direct continuity with the cranial cavity. Thirdly, it may be that the changes in intracranial pressure in the erect posture are a reflexion of the changes in arterial blood pressure. When simultaneous records of the direction of changes of intracranial pressure and arterial blood pressure are taken, it is observed that the changes in intracranial pressure are in the same direction as those of the arterial blood pressure. The time relationship between the two is so close that it is difficult to escape the conclusion that the changes of intracranial pressure in the erect posture on taking a deep breath are a reflexion of changes in arterial blood pressure. This view is supported by the fact that if the arterial blood pressure is caused to rise, as by a mathematical problem, a coincident rise in intracranial pressure occurs. It is also true that the fluctuations in intracranial pressure during quiet respiration in the erect posture are in phase with changes in arterial blood pressure.

There is still another possibility which might account for the changes in intracranial pressure, namely, active dilatation and constriction of the blood vessels within the cranium. It has been demonstrated that in association with a deep breath there is first a rise in arterial blood pressure and then a fall, and the fall occurs during the period when a diminution in finger volume is being recorded. The changes in finger volume have been shown by Goetz [1935] and by Bolton *et al.* [1936] to result from vaso-constriction of the finger blood vessels. It is possible, therefore, that the fall in intracranial pressure is consequent upon an active vaso-constriction of the cerebral blood vessels. Several observations are against such an hypothesis. Similar changes in intracranial pressure occurred before and after bilateral stellate ganglionectomy. As the vaso-constrictor fibres, if there be any to the cerebral blood vessels of man, are supposed to travel through the stellate ganglion to the superior cervical ganglion, these must have been interrupted when the stellate ganglia were removed. Yet, after bilateral stellate ganglionectomy, a fall of intracranial pressure occurred

on taking a deep breath. Thus the fall in intracranial pressure could not be dependent upon the activity of the vaso-constrictor nerve fibres to the cerebral blood vessels. Again, it has been shown that when a stimulus, such as the light application of a small piece of ice to the skin of the trunk or limbs, is given, vaso-constriction occurs in the finger vessels, without any associated change in intracranial pressure, provided there is no change in arterial blood pressure. Also, it has been shown that with mathematical exercise, vaso-constriction of the finger vessels occurs, but a rise in intracranial pressure takes place. It is appreciated that the occurrence of vaso-constriction in the peripheral blood vessels does not necessitate a similar constriction in cerebral blood vessels. It is of interest to note, however, that changes of fluid pressure take place in the same direction as blood-pressure changes, either a rise or a fall when the latter accompany vaso-constriction in the peripheral blood vessels. Lastly, actual stimulation of the stellate ganglion by an induced current after its central connexions have been severed causes a profound vaso-constriction of the finger vessels but no alteration of the spinal fluid pressure. For these several reasons it is not possible to account for the fall of intracranial pressure as the result of active vaso-constriction of cerebral blood vessels. It is also unlikely that the rise in intracranial pressure is the result of active vaso-dilatation of the cerebral vessels; the close time relationship to the changes of arterial blood pressure is too prominent.

This study, therefore, brings forward evidence indicating that in the erect posture of man the changes in intracranial pressure associated with quiet respiration, the taking of a deep breath or following mental exercise, are a direct reflexion of changes in arterial blood pressure. Roy & Sherrington [1890] postulated that the changes in cerebro-spinal fluid pressure were partly dependent upon arterial blood-pressure changes. Lewis [1908] showed that in one subject the changes in pressure obtained from a decompression opening were "not necessarily of venous origin". Although in the skull the fluid pressure changes are based on changes in the arterial pressure, the pressure changes in the isolated lumbar sac still reflect changes in the venous pressure. When the cistern and lumbar sac are in normal communication the pressure changes within the cranium brought about by arterial blood-pressure changes blot out the pressure alterations in the lumbar sac which result from the fluctuations in venous pressure.

It is apparent from this study that in the erect posture the intracranial pressure is not influenced by alterations of intrathoracic pressure, so that in healthy man intracranial pressure is kept relatively constant

and the cerebral circulation less open to fluctuations from alterations in venous pressure.

The results obtained in these investigations indicate further that if in man there are vaso-constrictor fibres to the cerebral blood vessels, they play little or no part in controlling the circulation of the brain under normal conditions. With the stimuli used it has not been possible to obtain any evidence of active vaso-constrictor elements going to the cerebral blood vessels.

A few observations dealing with the changes in intracranial pressure in subjects when horizontal have been made. Full consideration will be given to these in a future communication.

Summary

It has been shown that in the erect posture of man the alterations in intracranial cerebro-spinal fluid pressure on quiet respiration and on taking a deep breath coincide in time and direction with alterations in arterial blood pressure. The increased negative pressure in the chest during the inspiratory phase of a deep breath and on quiet respiration is not transmitted along the jugular vein to within the cranium.

Evidence has been brought forward which does not favour the control of the cerebral circulation of man by vaso-constrictor elements arising in or passing through the stellate ganglia and thence to the cerebral blood vessels.

The work described in this paper was undertaken on behalf of the Medical Research Council.

It is a pleasure to record our thanks to the Honorary Staff of the National Hospital for kindly permitting the study of patients admitted under their care. We are especially grateful to Mr Geoffrey Jefferson for permission to take records during the operation of stellate ganglionectomy.

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THE PITUITARY GLAND AND THE CONTROL OF URINARY SECRETION

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The relative parts played by the hypothalamus, anterior and posterior lobes of the pituitary gland in the control of urinary secretion have been investigated by many workers with conflicting conclusions. The more important experimental findings may be briefly summarized.[1]

Ranson and his colleagues have shown in a large series of cats that a lesion causing bilateral interruption of the supraoptico-hypophyseal system of nerve connexions is followed by:

(*a*) An immediate transitory polyuria of a few days' duration. This usually occurs but not in all cases.

(*b*) Permanent diabetes insipidus after a latent period of 8–15 days.

(*c*) Atrophy and degeneration of the posterior lobe of the pituitary [Fisher *et al.* 1935].

(*d*) Loss of the anti-diuretic and pressor substance from the posterior lobe [Fisher & Ingram, 1936; Ranson, 1937].

Similar results have been obtained in monkeys (Fisher *et al.* 1936] and in dogs [Broers, 1933].

Richter, working with rats, has found that:

(*a*) Hypothalamic lesions produce permanent diabetes insipidus [Richter, 1930].

(*b*) Separation of the pituitary stalk leaving the gland intact results in diabetes insipidus.

(*c*) Complete hypophysectomy is followed by a transitory polyuria but the permanent condition is produced if the posterior lobe is removed and part of the anterior lobe left intact [Richter, 1934]. This has been confirmed in cats by Ingram & Fisher [1936].

[1] The posterior lobe of the pituitary is used throughout this paper to designate the *pars intermedia* and *pars nervosa*.

Camus & Roussy [1913] showed that polyuria followed experimental hypothalamic lesions in animals which had previously been completely hypophysectomized.

The actions of extracts of the pituitary gland briefly are:

(*a*) Posterior lobe extracts containing the anti-diuretic principle inhibit the secretion of urine in unanæsthetized animals.

(*b*) Suitable extracts of the anterior lobe may cause a marked increase in urine excretion in intact animals [Teel, 1929; Barnes *et al.* 1933].

While investigating the relation between the posterior lobe of the pituitary and gastric secretion [Cutting *et al.* 1937], a large series of cats and rats were hypophysectomized. Since this presented an opportunity of following the urinary changes in both species under comparable conditions in one laboratory, detailed observations have been recorded. From a few preliminary experiments it was obvious that the characteristics of the immediate and delayed polyuria varied in the different species. As the ætiology of these also appeared to be different the results will be discussed under separate headings.

Results

Rats

In these experiments Wistar white rats have been used of about 200 g. weight. Water intake and urine output were measured daily. The diet while on the test consisted entirely of dried food compressed into cakes [Thomson, 1936]. Hypophysectomy was performed under ether anæsthesia by the retropharyngeal route. The completeness of the operation was judged by weight curves of the rats, interruption of the œstrus cycle in females and at post-mortem by inspection and sectioning when necessary of the sella and endocrine organs.

Immediate post-operative polyuria. Following a pituitary operation in a series of forty-five rats, polyuria was produced in forty animals. In most cases the gland was completely removed, but in three rats a large piece of anterior lobe remained intact. The secretion of urine commenced within 2 hours of the operation and occurred independent of fluid intake. The urine output in 24 hours may reach as high a level as 0·7 c.c. per g. body weight and the water intake 1·0 c.c. per g. weight: the normal values being approximately 0·02 c.c. and 0·1 c.c. respectively. These results, similar to those of Richter [1934], are given in Table I. The average excretion of urine of twenty-five rats from 1 to 4 hours after the operation is given and the maximum and minimum values included.

As none of these animals received water to drink during this period, the polyuria must be primary and the polydypsia secondary [Richter, 1935]. The average fluid exchange for the 24 hours' post-operative period of these rats is also shown. The urine was of low specific gravity 1004–1008, and did not contain any abnormal constituents.

TABLE I. Average urine output and fluid intake of twenty-five rats following a pituitary operation

	Hours post-operative				
	1	2	3	4	24
Total urine output (c.c.)					
Average	0·03	0·9	2·2	5·0	31·2
Maximum	0·5	2·9	5·0	8·0	79·0
Minimum	0·0	0·0	1·3	2·1	10·0
Water intake (c.c.)					
Average	0	0	0	0	51·7
Maximum	—	—	—	—	105·0
Minimum	—	—	—	—	22·0

The intensity and duration of the polyuria varies widely in different animals. The peak of fluid exchange generally occurs some 24–48 hours' post-operative. The return to normal may only take a few days, or the volumes fall gradually and then continue permanently at a raised level. Fig. 1 is a chart showing a small increase in fluid exchange which returned to normal limits in 5 weeks.

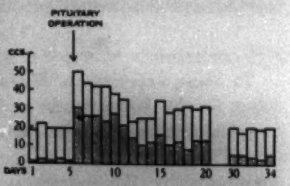

Fig. 1. Transitory polyuria in a rat after a pituitary operation—complete hypophysectomy.

Five of the forty-five rats operated on did not show any polyuria; of these three had a complete removal of the gland and two had an incomplete operation.

Persistent polyuria. Twenty rats have been observed for periods from 1 to 12 months after the pituitary operation. Eleven of these showed a normal urinary exchange and at autopsy were found to be completely hypophysectomized.

In nine animals, however, a significant persistent polyuria occurred (two of these did not exhibit any transitory effect). Four of these rats were found to have a large piece of anterior lobe tissue intact similar to those described by Richter [1935]. In Fig. 2 a chart of one of these is given. A marked transitory polyuria, which gradually diminished, was produced, but a raised urinary output was still present 145 days later.

Table II shows the fluid exchange of the four animals averaged for 7 days from the time, post-operative, indicated in the table. The detailed findings which are also given clearly indicate that these rats had functioning anterior lobe tissue.

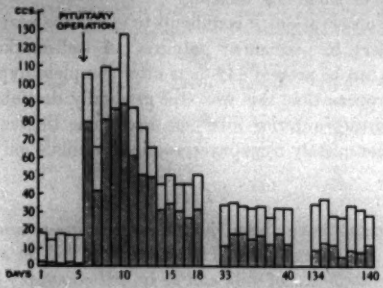

Fig. 2. Fluid exchange in a rat after removal of the posterior lobe of the pituitary.

TABLE II. Polyuria in four rats after removal of the posterior lobe with functioning anterior lobe tissue

	Days	24 hr. water intake c.c.	24 hr. urine c.c.	Body weight gain g.	Adrenal weight mg.	Ovary weight mg.	Œstrus cycle
HRt 210	105	32·0	10·8	+20	23	23	Positive
HRt 233	142	98·3	69·8	+1	23	14	Positive
HRt 186	145	31·0	9·8	+16	19	—	—
HRt 124	390	21·0	10·2	+17	33	40	Positive

Five of the rats, however, with a persistent polyuria were found to be completely hypophysectomized. Even 142 days' post-operative a marked increase in fluid intake was observed. These results are given in Table III.

TABLE III. Polyuria in five rats with absence of posterior lobe and anterior lobe tissue

	Days	24 hr. water intake c.c.	24 hr. urine c.c.	Body weight loss g.	Adrenal weight mg.	Ovary weight mg.	Œstrus cycle
HRt 195	64	20·0	11·6	−24	4	6	Negative
	121	17·0	8·0				
HRt 218	105	58·0	26·5	− 2	8	5	—
	142	38·0	14·5				
HRt 204	64	29·0	15·0	−50	5	—	Negative
HRt 209	105	25·0	11·8	− 1	11	4	Negative
HRt 221	142	58·5	30·8	−10	11	5	Negative

In two of these animals it is seen that the polyuria was gradually decreasing, but was still abnormally high at the time of autopsy. The associated findings are typical of a completely hypophysectomized animal, and the difference in adrenal and ovarian weights when compared with those seen in Table II is striking.

From these observations it is difficult to assess the part played by the anterior pituitary in permanent polyuria. A well-marked increase in fluid exchange can be present 142 days after complete hypophysectomy, but it would appear that this was still gradually declining. Every rat having a persisting anterior lobe has had some degree of prolonged polyuria, but completely hypophysectomized animals only show this in some cases.

Cats

These animals have been hypophysectomized under nembutal anæsthesia by the buccal route. They have been fed on a constant diet of 100 g. of meat, 150 c.c. of milk and an unlimited supply of water daily. Measurements of urine and food and fluid intake were made every 24 hours. In some experiments an unlimited supply of milk was also given.

Immediate post-operative polyuria. In the first experiments on cats the animals were given an unlimited supply of fluid to drink. In Table IV the effect of the pituitary operation is seen on the urine excretion of twelve animals. The average urine output for a 5-day period before the operation is given, and also the average excretion for the next 5 days following operation.

TABLE IV. Immediate effect of pituitary operation on twelve cats

Cat	Average 5-day urine, control c.c.	Average 5-day urine, post-operative c.c.
1	173	50·0
2	110	50·6
3	92	101·6
4	81	104·5
5	134	115·5
6	115	117·8
7	130	139·3
8	140	162·4
9	127	169·2
10	114	179·4
11	110	185·0
12	146	202·8

In four other animals an attempt was made to cut the pituitary stalk by the buccal approach. In this procedure the gland was freely exposed

and subjected to considerable manipulation and trauma during the operation. Table V shows the effect of the operation on the fluid exchange of these cats. These animals each received 150 c.c. of milk daily and 100 g. of meat. An unlimited supply of water was also given.

TABLE V. Transitory polyuria in four cats following a pituitary stalk operation

	Cat A		Cat B		Cat C		Cat D	
Days	Fluid intake c.c.	Urine c.c.	Fluid intake c.c.	Urine c.c.	Fluid intake c.c.	Urine c.c.	Fluid intake c.c.	Urine c.c.
1	255	150	155	100	180	100	150	110
2	235	85	155	124	210	120	150	55
3	180	127	160	115	210	120	150	126
4	150	105	150	85	165	90	150	135
5	150	70	150	100	120	100	160	100
Operation								
6	235	320	0	200	0	110	75	200
7	250	190	525	150	300	140	195	70
8	60	10	170	125	130	80	180	110
9	75	35	180	161	160	80	40	35
10	70	20	190	150	115	90	40	50

From these results it is seen that, following operations on the pituitary gland, a transitory polyuria frequently ensues. This, however, is slight, and neither the increase in fluid intake nor urinary output is comparable with the changes seen in rats following similar operations.

Permanent polyuria. Four completely hypophysectomized cats have been tested from 1 to 6 months after the operation to see if any permanent effect had been produced. In no case, however, was the urinary output found to be raised. Three other animals which had had an incomplete removal of anterior or posterior lobes similarly showed no abnormality. Owing to the anatomical situation of the posterior lobe it was not found possible to remove this lobe completely and leave the anterior intact.

In one cat, following an attempt to section the pituitary stalk, permanent diabetes insipidus was produced. This animal showed the typical changes as described by Ranson and his colleagues. A slight transitory polyuria followed the operation, but after a latent period of 20 days the urinary output greatly exceeded the fluid intake and the permanent condition commenced. The fluid intake continued at 400–600 c.c. daily and the urine excretion from 300 to 500 c.c. (the preoperative normals for this animal being 158 c.c. and 90 c.c. respectively). After 11 weeks a second operation was performed and the pituitary gland completely removed. The fluid intake and urine output immediately fell to a level slightly higher than the unoperated one. Further observations 10 weeks after pituitary removal showed that the normal unoperated

level was being maintained. A similar effect of hypophysectomy in diabetes insipidus in a dog was recently reported by Keller *et al.* [1936]. In a cat which has been described, the pituitary gland was obtained after the operation and assayed for the pressor and anti-diuretic principle. Whereas normal cat pituitaries have been found to contain from 4 to 6 pressor units per gland, this pituitary gave neither a pressor nor anti-diuretic response.

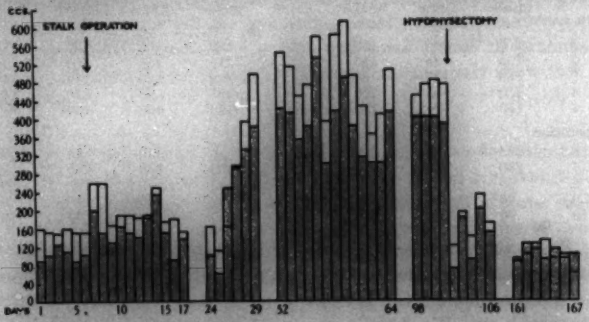

Fig. 3. Transitory polyuria, diabetes insipidus, and the effect of hypophysectomy in a cat.

Fig. 3 is a chart showing the changes described in this animal, namely, a transitory polyuria, permanent diabetes insipidus and the relief of this condition following hypophysectomy.

Discussion

The well-known anti-diuretic action of posterior pituitary extracts has suggested that this gland played a normal role in controlling urinary secretion. For many years, however, this has been doubted, since removal of the pituitary gland did not produce any lasting alteration of fluid exchange. The demonstration by many workers that permanent diabetes insipidus followed an experimental lesion in the hypothalamus tended to suggest that this area of the brain was the important ætiological factor rather than the pituitary gland.

The changes following lesions in the supra-optic nuclei have been carefully studied by Ranson, Ingram, Fisher and others. Their observations show that the changes in the posterior lobe of the pituitary are a degeneration and atrophy and a loss of the anti-diuretic and pressor principles. This suggested that hypothalamic lesions produced their

effect by the secondary changes in the posterior pituitary. The importance of the anterior lobe of the pituitary has been suggested by Richter. He has shown that rats with the posterior lobe removed do not develop diabetes insipidus unless functioning anterior lobe tissue is present.

The results which we have obtained on rats make it difficult to determine what part the anterior lobe plays in permanent diabetes insipidus. A raised urinary output can be present for 20 weeks after an operation in which the whole gland has been removed, but experiments have not been carried out long enough to see if this would diminish to a normal level. The long duration of the transitory polyuria, and the difficulty in distinguishing it from the permanent condition, makes the rat less suitable than the cat for this type of work.

Degeneration of the posterior lobe in a cat, with resulting absence of the pressor and anti-diuretic substance, produced diabetes insipidus. That this condition immediately ceased on anterior lobe removal suggests that, in this animal, the anterior lobe was essential for the maintenance of the permanent condition.

The way in which the anterior lobe may act is not clear. As Richter has suggested, either the principle which produces diuresis may act in the absence of the posterior lobe, or more simply, the general lowering of metabolism which occurs in the hypophysectomized animal may check the rapid fluid exchange in the body. In some preliminary experiments on hypophysectomized rats an attempt has been made to increase the metabolism by administering thyreotropic hormone or 4-6-dinitro-*o*-cresol, but so far it has been impossible to increase the fluid exchange by these methods. The rapidity with which the polyuria ceased following anterior lobe removal in the cat, which has been described, suggests that the lowering of metabolism, which would occur gradually, is not the true cause. The sudden withdrawal of a diuretic substance contained in the anterior lobe would appear the more likely explanation from our results.

True diabetes insipidus may therefore be considered as a hormonal deficiency of the posterior pituitary, while the exact part played by the anterior lobe is not yet fully established.

The transitory polyuria which occurs within a few days after operations on the pituitary or hypothalamus must be considered as a separate condition from the permanent polyuria. Considerable confusion has arisen in the literature because the transitory effect has been mistaken for the permanent condition.

The cause of the transitory polyuria is unknown, but various suggestions have been made. The diuretic effect of anterior lobe extracts

suggested that a liberation of this substance following trauma to the pituitary might cause the transitory effect. That this is not the explanation is seen from the experiments described. In rats the transitory effect is not related to the degree of pituitary removal, and is very marked. In these animals trauma to the pituitary is negligible when compared with the cats, where polyuria is small. Furthermore Teel [1929], Barnes *et al.* [1933] and Biasotti [1934] agree, from their studies of diuretic pituitary extracts, that a latent period of 3–5 days occurs before the maximum increase is attained. Also Teel states that no increase in urine volume occurs for 6–8 hours following injection. As we have shown, however, diuresis is marked 4 hours after a pituitary operation in rats.

Since complete removal of the pituitary in the rat is followed by a transitory polyuria in many cases, it does not seem possible to postulate any hormonal theory for its production. The only other explanation, therefore, is one of nervous stimulation. In support of this the following facts may be suggested: (*a*) The preliminary effect bears no relationship in intensity or duration to either the extent of pituitary removal or to the permanent condition. (*b*) The immediate polyuria is not comparable in rats and cats after the same extent of removal of glandular tissue. Anatomically, however, the relations of the pituitary gland are considerably different. (*c*) Transitory polyuria may be produced after complete hypophysectomy by a subsequent hypothalamic lesion [Camus & Roussy, 1913; Houssay *et al.* 1920]. (*d*) Repeated piqure in a dog has been shown by Warner [1931] to be followed each time by a transitory polyuria. The chief objections to a nervous theory for the production of transitory polyuria are raised by various workers who have produced polyuria after denervation of the kidneys [Houssay & Carulla, 1920; Camus & Gournay, 1923]. Further evidence that a hormonal factor may be responsible is given by cross-circulation experiments of Compère [1933] and Brull [1933]. At present it is impossible to conclude what causes the transitory polyuria following lesions in the hypothalamus or pituitary gland. It is certain that the pituitary gland does not play an essential part in its production, and therefore it is difficult to imagine what hormonal factor could produce the diuretic effect with such a rapid onset.

Since nembutal has been shown by Beattie [1932] to cause an inhibitory effect on hypothalamic nuclei, an attempt has been made to anæsthetize rats with this substance and then perform hypophysectomy. If the nervous stimulation produced by this operation was necessary to initiate the polyuria, it was thought that nembutal would abolish any

effect. Difficulty, however, has been experienced in keeping the animals under deep anæsthesia for 4 hours without mortality. In six experiments it appeared that animals deeply anæsthetized did not exhibit any polyuria, but as the anæsthetic passed off diuresis commenced.

Conclusion

Transitory polyuria usually occurs in the rat following hypophysectomy. This is independent of whether anterior or posterior lobes are removed.

In the rat posterior lobe removal results in a polyuria, which may be still present 140 days after operation, but this may also occur even in the complete absence of the anterior lobe.

The polyuria in rats differs in intensity, duration and ease of production from that in cats.

Permanent diabetes insipidus in a cat was permanently checked by hypophysectomy.

The cause of the transitory polyuria is unknown, but a hormonal mechanism would seem unlikely. Permanent diabetes insipidus is, however, of a hormonal nature, an inactive posterior lobe being necessary for its development.

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THE LOCAL ACTION OF ESERINE ON THE CENTRAL NERVOUS SYSTEM

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An antagonistic action of eserine (physostigmine) in relation to curare has been demonstrated by a number of investigators. Pal [1900] showed that eserine restores breathing and voluntary movements in animals previously curarized. Likewise after curare Rothberger [1901] observed that eserine re-establishes neuromuscular transmission in the sciatic nerve and concluded that eserine stimulates the motor endings in muscle. Langley & Kato [1914] obtained similar evidence of the power of eserine to diminish the effects of curare. This opposed action of eserine as regards curare is recognized by Dale *et al.* [1936] as a well-authenticated fact.

Recently Dale [1934] proposed the theory that acetylcholine serves as the transmitter between the motor nerve endings and the end-plates of voluntary muscle. The evidence in favour of this view was provided by the experiments of Dale *et al.* [1936] and by those of Brown [1937]. In accordance with these conclusions and with other current conceptions the stimulating action of eserine on the motor endings inferred by Rothberger may be supposed to be brought about through the inhibition of cholinesterase and the resulting lessened destruction of acetylcholine with a consequent augmented excitatory action on the muscle.

Closely allied to the action of eserine above described is a remarkable observation made by Brown *et al.* [1936], who found that the intravenous injection of eserine in the mammal exerts a potentiating influence on the muscular contractions evoked by maximal nerve stimulation. They favoured the interpretation of this result on the basis of the theory of chemical transmission just outlined, eserine inhibiting cholinesterase.

The view is widely held that the motor nerve ending and its end-plate have many properties in common with the synapse. Thus eserine might logically be expected to stimulate this latter structure, whether the action be direct or in consequence of the chemical process mentioned above. As a matter of fact Dale [1934] has suggested the possibility of transmission by acetylcholine at the synapse. Langley & Kato [1914] observed indications of stimulation of the central nervous system by eserine, and interpreted this result in harmony with prevailing views, by suggesting that the drug may increase the irritability of the synapses.

On the basis of the above considerations it occurred to me that eserine might reasonably be expected to have a local action on the cortex of the cerebrum and of the cerebellum, when applied directly. Accordingly, on 19 March 1935 (Exp. Es. 3. 35) I exposed the left motor cortex in a cat anæsthetized with chloroform and ether. Using faradism with bipolar electrodes a centre on the anterior sigmoid gyrus was localized from which flexion of the contralateral foreleg was evoked. Two small ophthalmic tablets of eserine salicylate (Burroughs, Wellcome and Co.), amounting together to 0·22 mg., were then applied in a small drop of saline to the above point and almost immediately the contralateral foreleg became rigid and in 5 min. showed extensor clonus. As a control the eserinized motor cortex was completely excised, whereupon the affected foreleg softened promptly. In another test the application of but a single tablet (0·11 mg.) to the cortical centre yielded promptly rigidity and extension in the contralateral foreleg.

It will be noted that while faradization of the centre yielded flexion of the limb the eserine application yielded extension. However, in other experiments, to be reported below, the eserine effect is flexion and simulates very closely that of faradization. Whether or not the eserine action is similar to that of faradization the circumstance that the incidence of both is in the same limb indicates clearly that eserine exerts a stimulating influence on the cortical centre.

Considering the large number and variety of synapses in the cerebellar cortex I next decided to test the local action of eserine on this structure. On 23 March 1935 (Exp. Es. 4. 35) a cat under deep anæsthesia was decerebrated with the Sherrington Decerebrator, the plane of section being such that the rostral surface of the lobus anterior of the cerebellum could be readily exposed by rongeur forceps. Under these conditions faradization of the cortex of the lobus anterior evokes inhibition of decerebrate rigidity in the fore- and hindlegs, neck, tail and other parts of the body [Sherrington, 1898], and it was later shown that this

reaction is aroused through local excitation of the cerebellar cortex [Miller & Banting, 1922]. Two ophthalmic tablets containing together 0·22 mg. eserine salicylate were now applied near the centre of the lobus anterior and almost at once the right foreleg was thrust forward, the left likewise but more slowly. The hindlegs relaxed promptly, then stiffened, later relaxed and stiffened alternately.

As in the case of the cerebral cortex the response to eserine differed in kind from that to faradism but here again the distribution of the effects of eserine and faradism was similar. It would appear indeed highly probable that eserinization reveals functions of the cerebellar cortex not made evident by faradism. Considering the small amount of eserine applied and the extreme promptness and definiteness of the response it must be concluded that the drug has a specific local action on the cerebellar cortex. As will be shown later, the mode of action probably consists in facilitation of transmission at synapses.

These demonstrations of the local stimulating action of eserine on the central nervous system were obtained incidentally in the course of other work and the conclusions were not at the time published. Recently Schweitzer & Wright [1937] reported that the intravenous injection of eserine augments the knee jerk and other reflex activities. The increased excitability of the spinal cord thus revealed they attribute to a property of eserine of facilitating synaptic transmission or to neuronal stimulation, possibly to both, and they regard this property of eserine as chemically distinct from its anti-cholinesterase action.

Methods

In the earlier experiments the cats were anæsthetized with chloroform and ether. In the later work dial was given by intraperitoneal injection, 0·3 c.c. per kg. of body weight, a small amount of chloroform being administered during the actual operation. Success in demonstrating the local action of eserine is dependent on the proper degree of dial anæsthesia. While the amount mentioned is suitable for a large cat, it sometimes appears excessive in the case of an animal of about 2500 g. In the latter case it was found advisable to expose the brain in a preliminary operation and make the eserine applications on the following day.

After preliminary tracheotomy the head was secured in a Czermak (Palmer) holder. The calvarium was opened up on one side, the anterior and posterior sigmoid gyri being fully exposed just behind the frontal

sinus; the excitability of the cortex was preserved by irrigation with warm saline. Localization was carried out by faradization with bipolar, beaded silver electrodes, the terminals of which were 3 mm. apart. The coil used was Palmer's H 32, operated by storage cells, with a suitable resistance in the primary circuit. At 15 mm. sec. dist. the current was just perceptible on the tip of the tongue.

In the preliminary experiments, reported above, the eserine was applied as small ophthalmic tablets. Since objection might be made that the eserine in this form is so concentrated as possibly to be irritating, the later applications were made by small squares of blotting or filter paper. These were 4 mm. on the side, thus having an area of 16 sq. mm. They were immersed in 1 or 10 p.c. eserine sulphate (Merck) in saline and were then applied to the specific cortical centres.

The approximate content of eserine sulphate in each square was calculated in the following way. It was found that 0·1 c.c. of saline was absorbed by 10 squares of blotting paper or 25 squares of filter paper, so that each square of blotting paper absorbed 0·01 c.c. of saline. In the case of the 1 p.c. solution, each square would thus contain 100γ eserine sulphate or for the 10 p.c. 1 mg. eserine sulphate. In the case of the filter paper, which was less often used, each square would contain 40γ eserine sulphate for the 1 p.c. or 400γ eserine sulphate for the 10 p.c. solution.

Results

Effects of eserine on motor cerebral cortex

Foreleg centre. The following protocols were selected from a number of experiments, in which essentially the same results were obtained:

Exp. Es. 10. 37. A cat weighing 2500 g. received an intraperitoneal injection of 0·75 c.c. dial; a small amount of chloroform was administered and the left motor cortex was exposed. Since the dial anæsthesia was profound the skull opening was closed by suturing the skin and the animal was left overnight.

On the following day at 10.50 a.m. faradism (12 cm. sec. dist.) was applied. In the lateral part of the anterior sigmoid gyrus a centre was localized, which yielded flexion and adduction of the contralateral foreleg (F_1, Fig. 1). In the middle of the posterior sigmoid gyrus was a centre for flexion of the contralateral hindleg (H). Both the above centres were later subjected to the action of eserine. A more medial centre in the anterior sigmoid gyrus for flexion and abduction of the contralateral foreleg was also localized (F_2), but this latter centre was not exposed to eserine.

At 12.18 p.m. a square of blotting paper soaked in 1 p.c. eserine sulphate was applied to foreleg centre F_1. 6 sec. after the application flexion began in the contralateral foreleg and increased progressively until 3 min. 30 sec., when slight tremors were perceptible in the limb. At 7 min. 35 sec. the limb was strongly flexed, retracted and adducted, and a slight clonus was present which gradually increased. The flexion and adduction were of the same nature as those from faradization of this centre, but were much more intense. Since the

clonus did not appear on preliminary faradization it must be regarded as a specific effect of the eserine. It was very striking: beginning as slight tremors, it steadily increased and finally revealed itself as beats of wide amplitude, resulting sometimes in powerful tapping of the table by the forepaw. The limb was felt to be rigid to passive extension, which itself

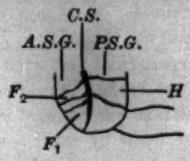

Fig. 1. Left motor cortex of cat in Exp. Es. 10. 37. Localization to faradism. *A.S.G.* anterior sigmoid gyrus; *C.S.* cruciate sulcus; *P.S.G.* posterior sigmoid gyrus; F_1, centre for flexion and adduction of contralateral foreleg; F_2, centre for flexion and abduction of contralateral foreleg; *H*, centre for flexion of contralateral hindleg. Eserine was applied to F_1 and to *H*.

greatly augmented the clonus, apparently as a stretch reflex response. At 9 min. 45 sec. after its application the blotting-paper square was removed from the cortex. The symptoms continued, however, for about 10 min. longer, after which they gradually subsided.

Prior to the eserine application, the faradic threshold for the centre was at 12 cm. sec. dist. At 10 min. after the application, faradism of this intensity yielded a much more extensive foreleg flexion with clonus and adduction, the entire response considerably outlasting the faradism and appearing as after-discharges. In addition, definite lowering of the cortical threshold was observed, since at 16 cm. sec. dist. strong flexion and clonus were evoked, though this strength of current was quite ineffective before eserinization of the cortex. In another trial the threshold was lowered from 12 cm. to 18 cm. sec. dist.

Thus the local eserinization of the motor cortex results in powerful facilitation to faradism, disclosed by lowered threshold and augmented motor discharges, accompanied by clonus. With the subsidence of the eserine effect the cortical threshold to faradism rose steadily until it equalled that before the application of the drug.

Hindleg centre. The effects of eserine application to the hindleg centre are quite comparable to those from the foreleg centre.

Exp. Es. 7. 37. A cat was anaesthetized by an intraperitoneal injection of dial, 0·3 c.c. per kg. The left motor cortex was exposed and a small area yielding flexion of the contralateral hindleg was localized by faradism. Approximately 3 hours after administration of the dial a filter-paper square soaked in 1 p.c. eserine sulphate was applied to the above hindleg centre. In 7 sec. tremors appeared in the lateral thigh muscles of the contralateral hindleg and the limb extended promptly.

In another experiment (Es. 12. 37) a centre was localized in the posterior sigmoid gyrus, yielding to faradism flexion of the contralateral hindleg, the threshold being at 13 cm. sec. dist. A blotting-paper square impregnated with 10 p.c. eserine sulphate was applied. In consequence tremors appeared in the lateral thigh muscles, the limb became rigidly flexed, then rigidly extended, all the muscles being apparently involved in the rigidity; the knee jerk was exaggerated and was accompanied by clonus. The faradic threshold

was lowered from 13 to 16 cm. sec. dist. The flexion was increased in amplitude by the original strength of faradism (13 cm.) and was followed by progression and clonus as after-discharges.

Thus marked facilitation to faradism resulted from eserinization of the cortical hindleg centre as in the case of the foreleg centre.

Discussion

Local action of eserine. That the eserine response is evoked locally from the cerebral cortex and is not a consequence of absorption followed by spinal or peripheral action is indicated by a number of considerations. In the first place the quantity of eserine sulphate applied in the square of filter or blotting paper was very small, as already indicated: thus in Exp. Es. 7. 37 the filter-paper square applied to the hindleg centre contained 40γ of eserine sulphate and in Exp. Es. 10. 37 the blotting-paper square contained 100γ. While these quantities were contained in the squares of filter or blotting paper, only a small proportion in each case would diffuse downwards to the surface of the cortex. The amount which would actually penetrate the pia and cell layers and finally combine with the synapses or act chemically would necessarily be extremely minute. Since, in order to produce generalized effects, at least 0·25–0·5 mg. eserine injected intravenously is required it is clear that the reactions observed are evoked locally in the cortex probably at, or in the neighbourhood of, the synapses. Considering the large number of synapses it is obvious that the quantity at each element must be infinitesimally small. Thus the eserine response appears to be highly delicate and specific.

Latent period. The latent period from the time of application of the eserine to the cortex is frequently in the neighbourhood of 6 or 7 sec., which periods are clearly much too brief to permit of production of the symptoms through absorption and circulation of the drug. Evidence against a generalized action of eserine is found in the normal quality of the posture and tone of the limbs not controlled by the eserinized cortex; thus there is no effect of eserine on spinal or other centres apart from those directly affected in the cortex. For similar reasons any action of eserine on motor nerve endings may be excluded. Further, salivation is absent, while the pupils react to light, even at the conclusion of a long experiment.

Faradic responses normal from non-eserinized cortex. The most conclusive proof of the absence of effects due to eserine absorption was afforded by faradic stimulation of the normal (right) motor cortex in

the experiment reported above, Es. 10. 37, in which the cortex was only exposed as a concluding procedure and hence there was no possibility of accidental direct contamination by the drug from the eserinized side. On applying faradism it was found that the centres for fore- and hindleg corresponded to those of the left side both in anatomical position and in the original threshold of excitability, and further, the type of response of each limb was exactly like that from the left side before the use of eserine.

Since the effects emanating from the eserinized (left) side had now largely disappeared 10 p.c. eserine sulphate was again applied to the foreleg centre on that (left) side. In 3 min. faradism (11 cm. sec. dist.) again yielded the typical eserine response to faradism, namely, flexion, clonus and adduction in the contralateral foreleg, the clonus appearing characteristically as an after-discharge. The foreleg response from the right cortex was, however, entirely normal, being moderate in extent and devoid of after-clonus. Thus it is clear that no effects of eserine absorption were revealed from the right cortex.

During the normal right-sided response to faradism, there was a curious secondary response in the foreleg controlled by the eserinized centre, consisting in extension, clonus and after-extensor clonus. This effect may perhaps be explained as follows: faradization of the normal centre evokes (with normal contralateral flexion) ipselateral extension, and proprioceptive impulses aroused in the extended muscles then travel through the hyperexcitable arcs of the eserinized cortex with a reverberation back to the muscles, thus enhancing their tone and clonus.

Effects of faradization and eserinization of cortex compared. As indicated in the experiments cited, the effects of eserinization of the cortical centres are sometimes similar to those of faradization, which mainly evokes flexion, but sometimes the eserine response is largely extension. One gains the impression that under eserine all the muscles of the limb are involved, with predominance now of one group, now of another. As will be explained later, the action of eserine is probably to facilitate conduction at synapses. In consequence, it cannot be expected precisely to imitate the effects of faradism. One could understand that it might sometimes do so to a degree, but a divergent or reversed effect is quite understandable, owing to its special pattern of selectivity at the synapses.

Variations of eserine effects from those of faradism are very evident in the experiment on the cerebellar cortex (Es. 4. 35) quoted in the introduction, but the extent of muscular involvement is similar in both cases. As already suggested, eserine probably discloses potentialities of

cerebellar action not shown by faradism. Thus the effects of eserinization of the cerebral and cerebellar cortex, whether resembling or differing from those of faradism, have a similar muscular distribution, and hence it is clear that eserine exerts a specific local action on the cortex.

Facilitation to faradism of eserinized cortex. The facilitation and augmentation of the response to faradism of the eserinized cortex was recorded in Exps. Es. 10. 37 and Es. 12. 37; the cortical threshold may be lowered from sec. dist. 12 cm. to 18 cm. Further, the amplitude of flexion of the limb is greatly increased, while there is associated clonus, both flexion and clonus continuing as after-discharges. These flexor responses to faradism are similar in appearance to those from eserine alone and may be regarded as intensifications of the local reactions, accentuated by the play of faradism. Both lowered threshold and increased responses to faradism of the eserinized cortex may probably be attributed to facilitated synaptic conduction.

Eserine and autogenous cortical activity. The doctrine is definitely established that many neurones habitually discharge rhythmically quite apart from the influence of afferent impulses [Adrian, 1934]. This autogenous activity, as revealed by electrical rhythms, is manifested in particular by cells of the cerebral cortex. Thus in the cerebrum, largely deafferented by section through the midbrain, Bremer [1935] observed powerful cortical potential changes. Even in the completely extirpated brain of the frog Gerard & Young [1937] found that electrical rhythms persist for long periods. As possible underlying causes of the electrical activity Davis [1936] suggests subliminal depolarizations of cell bodies, induced by chemical mediators at afferent synapses.

That the cortical potential changes are frequently not associated with detectable efferent discharges is generally recognized [Gerard, 1936]. It appears now highly probable that eserinization of the cortex enhances the autogenous rhythm of the neurones and thus makes it evident in tangible motor effects. Conceivably this may happen by facilitating transmission through the afferent synapses in a manner like that suggested by Schweitzer & Wright [1937]. The augmented cortical activity finds expression in the powerful muscular contractions and clonus, described as characteristic of the eserine response. Clonus depends on synchronism of the discharges of a large number of neurones [Creed *et al.* 1932] and is a condition brought about by facilitation at synapses. The cortical facilitation after eserine is probably similar to its potentiating effect at or near the nerve endings, as reported by Brown *et al.* 1936].

Summary

1. Eserine sulphate in a strength of 1 p.c. and occasionally of 10 p.c. in saline was applied to the cerebral motor cortex of the cat under dial by means of small squares of blotting or filter paper. The quantities applied were of the order of 40–100γ, of which only a small proportion would diffuse down to the surface of the cortex and the quantities which would penetrate the cortical layers would be very minute.

2. After a latency of about 6 sec. the contralateral fore- or hindleg shows tremors and soon develops a general muscular rigidity together with a powerful clonus.

3. The effects of eserinization of a cortical centre are focused in the same limb as the effects of faradization of that centre. Thus the action of eserine is localized and specific.

4. That the effects of eserine are produced locally and not by generalized absorption is shown by:

(*a*) the minute amounts which are effective;
(*b*) the shortness of the latency, about 6 sec.;
(*c*) the normal posture and tone of the remaining limbs;
(*d*) absence of salivation;
(*e*) presence of pupillary reactions to light;
(*f*) normal responses to faradization of the non-eserinized cortex.

5. Eserinization of the motor cortex results in facilitation to faradism, the threshold is notably reduced, while the responses are augmented and are accompanied by powerful clonus with prolonged after-discharges.

6. The local eserine reaction and the facilitation to faradism are believed to depend on enhanced transmission at cortical synapses. This explanation is in accordance with that given by Schweitzer & Wright for the increased knee jerk following the intravenous injection of eserine. The enhanced synaptic transmission is probably closely allied to the potentiating effect of eserine at or near the motor nerve endings, as recorded by Brown *et al.* [1936].

7. The hypothesis is proposed that the local eserine reaction with facilitation at synapses renders apparent the autogenous rhythm of the cortical neurones. This rhythm otherwise is revealed only by the cortical action potentials.

8. A few preliminary observations were made on the application of eserine to the cortex of the lobus anterior of the cerebellum. Prompt muscular effects in limbs and body were evoked, suggesting that eserine acts here also by facilitating conduction at a multitude of synapses.

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THE SECRETION OF URINE IN MAN DURING EXPERIMENTAL SALT DEFICIENCY

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It has been recognized by clinicians for some time that the blood urea may rise to very great heights in diseases which are not "nephritis" in the usual sense of the term. This is particularly true of diseases in which there is a fall in the plasma electrolytes, particularly the chlorides. The causal connexion, if any, is still far from clear and very few attempts have been made to solve the matter experimentally. Hitherto, no experimental work has been done on man. The subject was reviewed by Kerpel-Fronius [1936] and McCance [1936*b*] and no great advance in knowledge appears to have been made during the past year. It will only be necessary, therefore, to refer to papers with a direct bearing on the matter in hand.

In the present investigation five normal male adults have been made salt deficient by diet and sweating [McCance, 1936*a*, *b*]. Their kidney function has been studied before, during, and after the deficiency, but the tests employed have varied somewhat from one subject to another. This will be made clear in the presentation of the results. The subjects were not confined to bed while tests were being made, but their activity was restricted to quiet movement in the laboratory. The chemical methods were the same as those employed by McCance & Widdowson [1937]. Cane sugar, which was not used in that investigation, has been determined in the same way as inulin.

Results

(1) *The blood ureas*

During the experiments on R.A.M. and R.B.N. blood was collected almost every day at about noon for the determination of the blood urea. The findings are shown in Fig. 1. It is perhaps well to emphasize that

although the salt deprivation was being brought about by sweating, and although salt deficiency in man is accompanied by anhydræmia, both of these subjects were drinking enough water to maintain their urine volumes at or above their normal levels. A diminished urine flow, therefore, was not the cause of the rise of the blood urea. It will be observed that during the recovery period the blood urea fell below its original level.

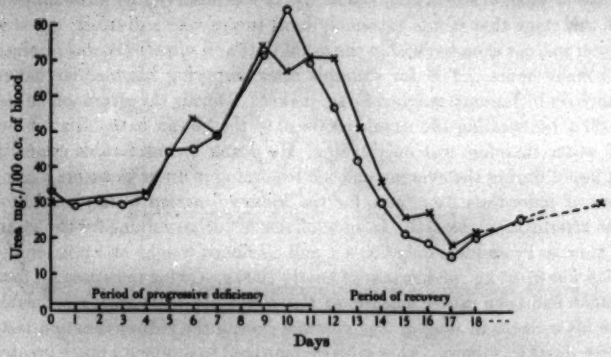

Fig. 1. Behaviour of the blood urea during and after salt deficiency.
o—o R.B.N. ×—× R.A.M.

This has been observed in the other subjects and is probably part of a general tendency of the body towards overcompensation during the recovery period. It is certainly not due to a diminished protein intake, and should rather be compared with the fall in the hæmoglobin and red blood cells to subnormal values which has always been found at about the same time [McCance, 1937].

(2) *The concentration of urea in the urine*

No deliberate attempt was made to measure the ability of the kidney to concentrate urea or to produce a urine of high osmotic pressure. The observations which have been made suggest that its ability was not impaired. R.A.M. on several occasions had concentrations of urea of 3·8–4·35 p.c. in his urine when he was salt deficient and higher concentrations have not been observed in this subject even when normal. One of R.B.N.'s 24 hr. urines, which had an unusually small volume, contained 3 p.c. of urea, and J.T.B. passed a specimen of urine containing 3·96 p.c. of urea when his blood urea was over 80 mg./100 c.c.

(3) *The excretion of water*

It became evident early in the investigation that the water metabolism of the salt deficient subjects was not normal. In the induction of a state of salt deficiency the removal of salt from the body was always followed by a loss of weight (presumably water). When the deficiency was well developed, however, the removal of further salt did not as a rule lead to a loss of weight [McCance, 1936*a*, *b*]. It was observed by most subjects at this stage that it was extremely hard to provoke a diuresis; ingested water was not excreted within the usual time and sometimes was retained for many hours. J.T.B. for example after emptying his bladder before lunch on 16 January weighed 87 kg. (naked). During the afternoon he lost 2080 g. by sweating and drank 1000 c.c. in the hot air bath. His net loss of water therefore was only 1080 g. He drank a considerable quantity of liquid during the evening and next morning in order to ensure a urine flow of more than 2 c.c./min. for the kidney function tests planned for the afternoon of the 17th. In spite of this his urine volume for the 24 hr. (1 p.m. to 1 p.m.) was only 550 c.c. and his naked weight at 1 p.m. on the 17th was 87·86 kg., an increase of nearly 1000 g. Owing to nausea his food intake had been rather meagre, so this could not have been responsible for his increase in weight. The volumes during the kidney function tests were about 1 c.c./min. Other subjects managed to produce a much greater diuresis than this, but the highest rate of flow recorded in these salt deficient subjects has been 5·83 c.c./min. Minute volumes of more than 12 c.c. could always be obtained with ease when the subjects were normal. These results corroborate those of Baldes & Smirk [1934] who found that 1 litre of water provoked a subnormal diuresis in human subjects who had been made salt deficient. These authors employed diet and sweating to produce the deficiency which, however, was probably much less severe than that in the present subjects.

(4) *The urea clearances*

In the first two experiments (on R.A.M. and R.B.N.) a 2-hourly specimen of urine was collected almost every day before lunch, no attempt being made to produce a large diuresis. The clearances were calculated as a percentage of "normal" by the standard or maximum formula and their course is shown in Fig. 2. It will be noted that R.B.N.'s preliminary clearances were well over 100 p.c., they fell to 40–60 p.c. of normal during salt deficiency, and returned to their original level at the end. R.A.M.'s were about 100 p.c. at first, they fell to about 70 p.c. and passed through a supernormal phase after the deficiency period was over. R.A.M.'s

clearances fell to much lower levels than those shown in Fig. 2 (32–36 p.c. of normal) during the 3–5 hr. following sweating in the hot air bath. His daily excretion of urea was much lower than it should have been considering the level of urea in his blood. This was demonstrated by

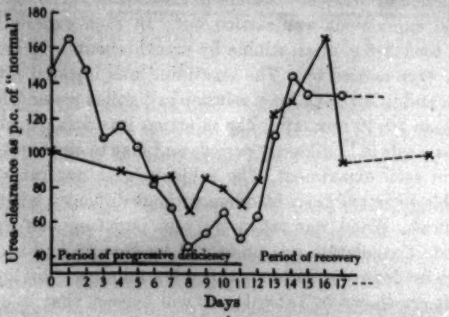

Fig. 2. Behaviour of the urea clearances (expressed as a percentage of normal) during and after salt deficiency.

o—o R.B.N. x—x R.A.M.

another experiment in which the subject ate about 250 g. of protein a day for 5 days. This raised his blood urea to similar levels, but also increased rather than decreased his urea clearances (Table I). Presumably

Table I. A comparison of R.A.M.'s urea clearances while salt deficient and while on a very high protein intake

During salt deficiency		During a very high protein intake	
Blood urea mg. per 100 c.c.	Urea clearance p.c. of normal	Blood urea mg. per 100 c.c.	Urea clearance p.c. of normal
53	84	53	107
67	79	59·5	128
71·5	70	70·0	106

Cope [1933] failed to lower the urea clearances by reducing the salt content of the body because his measures to effect the latter were comparatively mild.

In the other subjects urea clearances were not determined daily but only during the times when the creatinine, sucrose and inulin clearances were being carried out. These will now be described.

(5) *Clearances of substances without significant "augmentation" limits*

(*a*) *Creatinine clearances.* These were determined on four subjects, R.B.N., D.W., J.T.B. and R.M.L. Two to six experiments were carried out on each subject. Normal experiments were performed before and after the period of deficiency, except in the case of J.T.B. on whom only one normal experiment was carried out. In each experiment R.B.N. and D.W. took 5–6 g. of creatinine by mouth about 1–1½ hr. before the clearances were carried out. The creatinine was injected intravenously into J.T.B. and R.M.L. as a 3 p.c. solution in distilled water. The collection periods began 20–40 min. after the injection was finished. Urines were collected as a rule in half-hourly periods and four to eight specimens were obtained in each experiment. The subjects were not catheterized but were all able to empty their bladders without difficulty when specimens were required. Blood was taken by vein puncture half-way through each period. Coagulation was prevented by heparin and the corpuscles were separated from the plasma within a few minutes of taking the blood. The results are shown in Table II. It will be seen that in each subject

TABLE II. The creatinine clearances during health and during salt deficiency

	Normal health			During salt deficiency		
Subject	No. of experiment	No. of clearance periods in each experiment	Clearance, c.c./min. Average and range	No. of experiment	No. of clearance periods in each experiment	Clearance, c.c./min. Average and range
R.B.N.	1	5	181 (153–210)	1	5	121 (78–147)
	2	5	189 (166–215)	2	4	142 (124–156)
	3	5	157 (145–190)	3	5	124 (107–152)
D.W.	1	4	200 (182–222)	1	5	128 (123–132)
	2	5	173 (150–193)	2	5	116 (90–128)
	3	6	145 (121–160)	—	—	—
J.T.B.	1	8	203 (178–235)	1	5	136 (120–145)
R.M.L.	1	6	184 (134–213)	1	4	148 (132–157)
	2	6	181 (168–202)	2	5	133 (123–152)

there was a considerable fall in the creatinine clearances during salt deficiency. In most cases there was no overlapping of the normal and salt deficient ranges.

(*b*) *Sucrose.* This substance was used as a guide to glomerular filtration rates on one subject only, D.W. At this time inulin had just been found to be extremely toxic [McCance, 1936*b*] and no method for its purification was available [Goldring & Smith, 1936]. 50 g. were given intravenously as a 25 p.c. solution in distilled water over a period of 30 min. The injection terminated at least half an hour before the collection

TABLE III. The cane sugar clearances of D.W. during health and during salt deficiency

Normal health			During salt deficiency		
No. of experiment	No. of clearance periods in each experiment	Clearance, c.c./min. Average and range	No. of experiment	No. of clearance periods in each experiment	Clearance, c.c./min. Average and range
1	4	105 (99–118)	1	5	76 (70–86)
2	5	112 (104–119)	2	5	76 (62–94)
3	6	118 (108–130)	—	—	—

periods began. Creatinine and urea clearances were determined simultaneously. The results are shown in Table III. It will be observed that there was a reduction in the sucrose clearances, similar in magnitude to that of the creatinine clearances (Table II).

(*c*) *Inulin clearances.* These were determined on J.T.B. and R.M.L. (simultaneously with the creatinine and urea clearances). 50 g. were given intravenously as a 25 p.c. solution in distilled water containing 3 p.c. of creatinine. The inulin was purified as suggested by W. H. Smith, J. A. Shannon & H. Chasis in a personal communication, and the batch used for J.T.B. was quite non-toxic. A fresh batch was prepared for R.M.L.'s experiment and this subject experienced a slight chilliness and discomfort for about an hour during his preliminary normal experiment.

TABLE IV. Inulin clearances during normal health and salt deficiency

	Normal health			During salt deficiency		
Subject	No. of experiment	No. of clearance periods in each experiment	Clearance, c.c./min. Average and range	No. of experiment	No. of clearance periods in each experiment	Clearance, c.c./min. Average and range
J.T.B.	1	8	147 (123–186)	1	5	112 (106–118)
R.M.L.	1	6	139 (107–156)	1	4	114 (93–123)
	2	6	137 (121–154)	2	5	93 (84–105)

The attack began about 60 min. after the inulin had been injected. The inulin was repurified and R.M.L. experienced no reaction following two equally large injections of this inulin when he was salt deficient. He had another mild reaction, however, during the last normal experiment even though the same repurified inulin was used. It is not easy to see why this should have happened but it seems advisable to place it on record although it is not thought to have had any influence upon the kidney function. The results are shown in Table IV, and again indicate a substantial fall of the clearances in both subjects during their periods of salt deficiency.

(6) *Clearance ratios*

With minute volumes of less than 2 c.c./min. the output of urea varies with the minute volume of the urine. The outputs of creatinine, inulin and sucrose do not. Thus clearance ratios involving urea vary with the minute volume when this is below 2 c.c./min. It was therefore originally intended to maintain the urine volumes above 2 c.c./min. throughout all the kidney function tests. This was found to be impossible owing to the difficulty of producing a satisfactory diuresis in several of the subjects. In order therefore to enable direct comparisons to be made of the clearance ratios during the normal and deficient periods attempts were made to carry out normal tests at minute volumes comparable with those produced during salt deficiency. This also proved difficult. Consequently, in order to compare the clearance ratios during the normal and deficient periods it is necessary either to use only the limited number of clearances available over comparable volumes of urine, or to use all the clearance periods after applying the appropriate formula to express the urea clearances as a percentage of "normal". Both alternatives have been tried (see Tables V and VI) and both give essentially the same results. The significant facts which emerge from a study of Table V seem to be: (*a*) that salt deficiency decreased the urea clearance/creatinine clearance ratio except between minute volumes of 1·48 and 2·1 in the case of J.T.B.

TABLE V. A comparison of the (true) clearance ratios during health and during salt deficiency

Subject	Range of min. vol.	No. of clearance periods averaged	Clearance ratios*				
			U/C	*U/S*	*U/I*	*C/S*	*C/I*
			Normal health				
R.B.N.	0·94–1·28	6	0·452	—	—	—	—
	1·5 –1·8	2	0·432	—	—	—	—
	2 –2·3	2	0·470	—	—	—	—
D.W.	2·26–5·8	7	0·445	0·676	—	1·52	—
J.T.B.	1·48–2·1	3	0·288	—	0·365	—	1·27
	1·17–1·32	1	0·326	—	0·450	—	1·38
R.M.L.	2·5 –4·14	3	0·445	—	0·568	—	1·27
			During salt deficiency				
R.B.N.	0·94–1·28	6	0·186	—	—	—	—
	1·5 –1·8	2	0·224	—	—	—	—
	2 –2·3	2	0·244	—	—	—	—
D.W.	2·26–5·8	9	0·365	0·586	—	1·61	—
J.T.B.	1·48–2·1	1	0·310	—	0·380	—	1·23
	1·17–1·32	2	0·254	—	0·308	—	1·22
R.M.L.	2·5 –4·14	4	0·310	—	0·405	—	1·30

* *U* = urea. *C* = creatinine. *S* = sucrose. *I* = inulin.

The validity of this exceptional ratio rests upon the results of a single test period and should possibly be questioned. (*b*) That salt deficiency decreased the urea clearance/sucrose clearance ratio and the urea clearance/inulin clearance ratio. Again, however, the ratios over J.T.B.'s minute volumes of 1·48–2·1 were exceptional. (*c*) That salt deficiency did not alter consistently the creatinine clearance/inulin clearance ratio or the creatinine clearance/sucrose clearance ratio.

Table VI shows that when the urea clearances are expressed as a percentage of normal, salt deficiency produced in every subject a fall in the ratios urea clearance/creatinine clearance, urea clearance/sucrose

TABLE VI. A comparison of all the creatinine, sucrose, inulin and urea clearance ratios. The urea clearances are expressed as a percentage of normal

Subject	No. of clearance periods averaged	Clearance ratios*				
		U/C	*U/S*	*U/I*	*C/S*	*C/I*
		Normal health				
R.B.N.	15	0·718	—	—	—	—
D.W.	15	0·596	0·895	—	1·50	—
J.T.B.	8	0·453	—	0·625	—	1·38
R.M.L.	12	0·575	—	0·763	—	1·32
		During salt deficiency				
R.B.N.	13	0·290	—	—	—	—
D.W.	10	0·493	0·790	—	1·60	—
J.T.B.	5	0·434	—	0·526	—	1·22
R.M.L.	9	0·420	—	0·575	—	1·37

* *U* = urea. *C* = creatinine. *S* = sucrose. *I* = inulin.

clearance and urea clearance/inulin clearance. J.T.B. showed the least change and R.B.N. the greatest. There was no significant change in the creatinine/sucrose or creatinine/inulin clearance ratios. This confirms the results shown in Table V, which were obtained from a much smaller number of clearance periods.

DISCUSSION

It is probable from the work of Shannon & Smith [1935] and Hendrix *et al.* [1936] that inulin clearances give a measure of the glomerular filtration rates, and if this is the case, some creatinine is normally excreted by the tubules and some sucrose reabsorbed. Be that as it may, the clearances of all three substances appear to be reduced in exactly the same proportions by salt deficiency. The urea clearance is also reduced by salt deficiency but to a greater extent than the creatinine, inulin, or sucrose clearances. It is difficult to understand why salt deficiency should bring about differential filtration rates in the glomeruli. One must suppose, therefore, that the fall in the clearance

ratios involving urea is due to increased urea reabsorption [Shannon, 1936; Gordon *et al.* 1937]. In other words Blum *et al.* [1928, 1929] were, to a limited extent, correct in postulating a reabsorption of urea "par manque de sel". Why salt deficiency should lead to an increased reabsorption of urea is a matter of pure conjecture since nothing is known about even the normal mechanism. The main cause of the "uræmia", however, would appear to be a reduction in the glomerular filtration rate. The present experiments show that salt deficiency does not reduce the glomerular filtration by causing a fall of arterial blood pressure, since the blood pressure of these subjects did not fall [McCance, 1936*b*]. The decrease in filtration rates in these experiments, moreover, is not due to a diminished protein intake [Cope, 1933; Pitts, 1935; Shannon *et al.* 1932; Goldring *et al.* 1934; Herrin *et al.* 1937]. It is true that J.T.B. was so nauseated while he was salt deficient that he ate little for 2 days except carbohydrate, but the level of the blood ureas in all the subjects and the fact that they were excreting 14–23 g. of N daily in the urine shows that their protein metabolism was high. The remaining possibilities seem to be: (*a*) an increase in the colloidal osmotic pressure of the blood (this certainly occurs in man, but may be unimportant, for in rabbits salt deficiency may lead to a fall in glomerular filtration without any concentration of plasma colloids [Wilkinson, 1937]). (*b*) A reduction in the number of "active" glomeruli owing to the diminution in blood volume. (*c*) A diminished circulation rate (caused by increased blood viscosity). (*d*) Something unknown and possibly unsuspected. It is necessary to make this reservation because of the fluctuations in glomerular filtration rates which seem to occur in beriberi [Nakazawa & Kusakari, 1930], or uncompensated alkalosis [McCance & Widdowson, 1937] and possibly following diabetic coma [McCance & Lawrence, 1935], or alkalosis during salt deficiency [McCance & Widdowson, 1936], etc., and for which none of the above explanations suffice. The work of Herrin *et al.* [1937] suggests that the rise and fall of glomerular filtration rates associated with variations in protein intake may be metabolic in origin. It is not at present clear why the metabolism of certain substances should affect the glomerular filtration rate, but the extension of this conception to the investigation of clinical problems seems to offer interesting possibilities.

SUMMARY

Severe salt deficiency produced experimentally by diet and sweating in normal men was accompanied by:

(*a*) A rise in the blood urea.

(*b*) No obvious change in the power of the kidney to concentrate urea.

(*c*) A diminution in the power of ingested water to produce a diuresis.

(*d*) A fall in the creatinine, sucrose, inulin and urea clearances. The clearances of the first three fell together and to the same extent. The fall in urea clearance was proportionately greater.

It is probable from a consideration of (*d*) that salt deficiency led to diminished glomerular filtration and also some additional urea reabsorption.

The authors are very grateful to the subjects for their co-operation in some rather uncomfortable experiments. One of us (E. M. W.) is indebted to the Medical Research Council for a part-time grant.

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OBSERVATIONS ON THE INACTIVATION OF ADRENALINE BY BLOOD AND TISSUES *IN VITRO*

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THOUGH knowledge relating to the chemical mediation of the effects of cholinergic nerve activity has advanced rapidly in the course of the last few years, the same progress has not been made with respect to our knowledge of adrenergic nerve activity. It appeared to us that one of the factors responsible for this latter state of affairs was a lack of information concerning the inactivation of adrenaline under experimental conditions resembling as closely as possible the conditions which obtain in the body.

The work recorded in this paper is an attempt to fill some of these gaps in our knowledge of adrenaline inactivation. It is necessarily incomplete and our interpretation of most of the phenomena described here for the first time must be considered tentative. Many fresh problems arose as the work proceeded and only a few of these have been solved: others we hope to deal with later.

We have worked for the most part from first principles. Our main object was to determine whether adrenaline is destroyed by blood and other tissues in a manner analogous to that in which acetylcholine is destroyed, and to determine whether cocaine produces its potentiating action on adrenaline effects, and on effects of adrenergic nerve stimulation, by preventing or delaying this destruction, in the same way as eserine potentiates the action of acetylcholine.

A chance observation, that the degree of inactivation of adrenaline in blood at the end of 10 hr. was no different from that at the end of 5, led us to investigate in more detail what happened to adrenaline when added to blood alone, and it is with this phase of our work that the second and major part of the present paper deals. The order of presentation of

our results may seem illogical in so far as our detailed description of what happens to adrenaline in blood follows our description of experiments with liver; but it is simpler to deal with the work in the order in which it was done and so, to this extent, we sacrifice logic to convenience.

Our experience of the bio-assay of adrenaline in the earlier experiments enabled us to develop a method of following the inactivation over periods of several hours, at such intervals, and with such a degree of accuracy, as to make the construction of inactivation-time curves possible. It is in the form of such graphs that most of our results are presented. Certain of our early and more or less qualitative experiments on the action of liver and cocaine have been repeated using this later technique, and the results are given in the same form as the others. Some of our results have been communicated to the Physiological Society [Bain & Suffolk, 1936; Bain *et al.* 1936].

The literature on adrenaline inactivation is scanty. For further information and references on the subject the reader is referred to the papers of Elliott [1905], Wiltshire [1931] and Blaschko *et al.* [1937].

Materials and methods

General details

In most of the experiments the medium in which we measured adrenaline inactivation was oxalated or defibrinated cat's blood, with or without added tissue slices or extracts. The blood was collected into clean or oxalated glass vessels from a cannula in the common carotid artery of a spinal or etherized cat. There was no apparent difference in the results with oxalated and with defibrinated blood, nor did it appear to matter whether the blood was obtained from spinal or from etherized animals. For experiments continued for 24 hr. or longer the blood was collected under conditions as nearly aseptic as possible, but usually no such precautions were taken.

The adrenaline used was the pure synthetic lævo base of British Drug Houses, Ltd. A concentrated stock solution of the hydrochloride was made by dissolving an amount of the base in a volume of $N/10$ HCl such that the solution contained 10 mg. adrenaline base per ml. From the concentrated stock solution we prepared, with distilled water, a dilute stock solution containing exactly 1 mg. of the base per ml. The experimental solutions in blood, in which inactivation was to be determined, were made up by adding measured quantities of the dilute stock solution to measured volumes of the blood. In experiments where several

different initial concentrations of adrenaline in blood were investigated, and in which therefore the volume of blood having a given concentration of adrenaline was small, the dilutions were effected by making up a certain volume of blood to an initial concentration of, say, 100γ/ml., and diluting measured portions of this to give the other desired and lower initial concentrations.

The standard solutions of adrenaline used for the assays were prepared from the dilute stock solution by further dilution with distilled water. The concentrations of adrenaline in the standard solutions were similar to the initial concentrations in blood, so that quantities of standard and experimental solutions of the same order were being measured during the assays, thus, to a large extent, obviating major errors of measurement. For injection of very small standard quantities of adrenaline in the late stages of an experiment some of the standard solution was still further diluted before use: such diluted standards were always checked against the original.

All the reactions described were carried out at a temperature of $38 \pm 1°$ C. except where otherwise stated. In most of the early experiments the experimental fluids were placed in test-tubes or hard glass flasks in a Wassermann bath and agitated at intervals to counteract sedimentation and promote full oxygenation. In all the later experiments the fluids were either in hard glass flasks or 25 ml. Jena glass vaccine bottles and were agitated continuously during the whole experiment.

Method of assay

The "adrenaline values" or "adrenaline equivalents" of the various experimental fluids were determined by blood pressure assay on cats, by what may be called the method of "continuous assay". In the earliest experiments we used both spinal and anæsthetized cats, but as the former proved much more sensitive to adrenaline all our later assays were made on spinal animals: all the results reported here refer to experiments on atropinized spinal preparations which had been "rested" for 2–3 hr. after transection of the spinal cord and destruction of the brain.

The arterial blood pressure was recorded from the left common carotid artery, and the anticoagulant fluid in the manometer system was a half-saturated solution of sodium bicarbonate. Injections were made into the right external jugular vein through a narrow metal cannula the proximal end of which fitted the nose of a record syringe: the cannula was provided with a stilette.

All the injections of experimental or standard solutions were made up to 1 ml. by dilution with Locke solution *immediately* before injection and were washed in with a further 0·5 or 1 ml. of Locke solution, making the volume of fluid injected at each observation 1·5 or 2 ml. according to the particular experiment. Control experimental fluids were treated in an exactly similar way. In some instances, where the amount of adrenaline left in the experimental fluids was small, or where the initial concentration was itself small, as much as 3 ml. of *undiluted* blood and adrenaline, or of control blood, had to be injected at an observation; but in general the total amount of undiluted experimental fluid measured out and subsequently diluted before injection was not more than 0·5 ml. and, in the early stages of an experiment, was usually less than 0·1 ml. These small amounts were measured into little conical glass dishes, and, a few seconds before the injection was due, were made up to volume with Locke solution from the injection syringe, rapidly mixed by barbotage, taken up and injected.

Injections were given at regular intervals of 3–6 min., the interval in any particular experiment depending chiefly on the time taken for the blood pressure to recover from a previous injection. After giving a number of injections of various doses of standard adrenaline, in order to determine the initial sensitivity of the animal, we continued with the injections of the standard doses within the range of greatest sensitivity. At convenient intervals a standard injection was replaced by an experimental one, the volume of experimental fluid taken up for dilution and injection being such as was calculated to bring the blood pressure rise between two of the standard rises, and as near to one of these as possible. Injections of standard and experimental fluids were continued in this way until the end of the experiment.

An important point about this continuous assay technique is that after 20 or 30 injections a large rise of blood pressure may be got with an amount of adrenaline which, at the beginning of the experiment, gave a small rise or no rise at all. This increase in sensitivity to adrenaline is always seen under the conditions of our experiments and usually occurs without any appreciable change in the resting blood pressure level. Sometimes the maximum increase may be only 80 p.c. for a given dose or it may be as much as 800 p.c. Whatever the extent of the increase it usually takes place smoothly, provided the injections are continued at regular intervals and that the heights of the blood pressure rises in response to the injections do not suddenly become much greater or much smaller, on the average, by marked alterations in the amounts of

adrenaline injected. The sensitivity generally reaches a maximum after 40 or 50 injections, may stay at this level for hours, and then decrease either gradually or suddenly. This decrease usually heralds the death of the animal, though in some instances there may be a fall after a few hours and the sensitivity persist at this lower level for a further period. The rate at which maximum sensitivity is attained seems to depend not so much on the number of injections given as on the blood pressure rises which they cause: the larger the individual rises of blood pressure the quicker is the rise of adrenaline sensitivity.

The importance of this change of sensitivity need not be stressed but it shows the absolute necessity for continued injections of standard adrenaline solutions throughout an experiment. It is also evident that if a high degree of sensitivity in the animal is desired before the assays proper are started, this can be achieved by giving a sufficient number of standard injections beforehand. For the type of work described here the increase of sensitivity is advantageous in that as the adrenaline disappears from our experimental solutions we are not compelled to increase the amount of such solutions injected to the same extent as would otherwise be necessary.

The standards to be injected are determined in the first place by the initial sensitivity of the animal and usually have to be modified later on: thus at the start of an experiment convenient standards may be 3, 3·5 and 4γ or more and, when full sensitivity is established, 0·5, 1 and 1·5γ or less.

Our method of arriving at the actual amount of adrenaline in the different volumes of experimental fluid injected is as follows: The blood pressure rises are measured and plotted on graph paper against time as abscissa. A line is drawn through each set of points representing rises for a particular dose of standard. The adrenaline value of any given experimental injection is then determined by observation or calculation.

As noted above, standards were not usually closer than 0·5γ, but in good experiments, where the sensitivity is high and the sensitivity curves are smooth, the adrenaline equivalent of the actual volume of fluid injected can be estimated to 0·1γ, especially if the position of intermediate points on the sensitivity curves is determined on the basis of a few injections and interpolated accordingly. In experiments where low concentrations of adrenaline had to be determined, standards differing by as little as 0·05γ were used.

A remarkable feature of these assay experiments is the large number of injections that can be given to a single spinal animal without causing

any appreciable change in the level of the resting arterial blood pressure, or in the accuracy of the adrenaline determinations. Most of our cats have had at least 60 injections and many well over 100, and it is very seldom that an experiment has had to be abandoned because of irregular responses at a late stage in the assaying. Bad cats are usually so from the start.

Results

Part I. Preliminary observations on inactivation by blood and tissues

In oxalated blood plasma and in blood serum at body temperature the inactivation of adrenaline proceeds to completion as it does in normal saline, Ringer or Locke solution. The inactivation proceeds in an almost

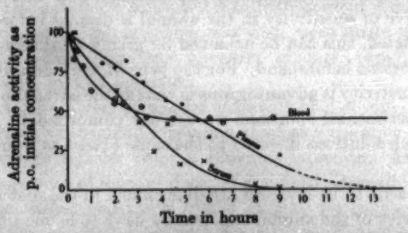

Fig. 1. To show rate and extent of inactivation of adrenaline at body temperature in (i) oxalated or defibrinated whole blood, (ii) blood plasma, and (iii) blood serum. Initial concentration of adrenaline 40γ/ml. in all cases. Data from Exps. A 47, 50, 58, 63 and 64.

linear manner during the greater part of its course and then tails off; it is more rapid in serum than in plasma. In oxalated or defibrinated blood inactivation of adrenaline does not proceed to completion: it proceeds in an exponential manner and ceases when an amount, which depends on the initial concentration of adrenaline, has disappeared. These facts are illustrated in Fig. 1, which shows the combined results from five typical experiments.

The difference between the behaviour of adrenaline in plasma and in serum seems to be chiefly one of rate. It is not accounted for by the absence of oxalate from the serum—since oxalated serum behaves in exactly the same way as normal serum—and it may be due simply to the difference in protein content of the two fluids. Comparison of the behaviour of adrenaline in whole shed blood, and in plasma or serum, shows that the difference here is of another order: it is a difference in kind. In shed blood, whether oxalated or defibrinated, it seems that inactivation

proceeds to what it is convenient to term an "equilibrium concentration" of adrenaline beyond which no further inactivation can be demonstrated.

It thus appears at the outset, from the marked contrast between the action of whole blood on adrenaline and its action on acetylcholine, that the hypothetical "oxidase" for adrenaline, corresponding to the known esterase for acetylcholine, either does not exist in the blood or, if it does, is incapable of exerting its full action under the conditions of our experiments. That we still get this equilibrium concentration even with full oxygenation of the blood and adrenaline shows that, apart altogether from the question of oxidizing enzymes, and apart, too, from the question of what has happened to the inactivated portion of the original adrenaline, full auto-oxidation itself is in some way prevented in whole blood.

Having failed to show complete inactivation of adrenaline by blood, we turned our attention to the possible effects of tissues added to blood and adrenaline *in vitro*.

Effects of tissues on adrenaline activation in blood. The tissues were removed from the animal used to supply blood for the experiment, cut into thin slices with a razor, rapidly washed to free them from blood and the excess moisture removed by filter papers, weighed, and added to the blood in the proportion of 1 g. of tissue/ml. blood. Adrenaline was then added to the mixture and to the control blood to give the same initial concentration per ml. of blood in each. Often the reverse procedure was adopted, i.e. 1 ml. of blood and adrenaline per g. tissue, mixed immediately beforehand, was added to the weighed liver slices. Control mixtures of tissue and blood were also set up and the different mixtures incubated in the usual way.

Of the tissues studied liver showed by far the greatest activity, and so attention was concentrated on it. Of the others, skeletal muscle was slightly active and kidney tissue intermediate between skeletal muscle and liver. Experiments on lung tissue proved fruitless because of the large amounts of an atropine-resistant histamine-like substance liberated during incubation.

That addition of liver slices to blood and adrenaline leads to the complete disappearance of adrenaline activity from the blood is shown in Fig. 2. This activity of liver is diminished by dipping the tissue in boiling water, and abolished by boiling for 2 min.

The activity of liver appears to be diminished by acid. This statement is based on two experiments (A 16 and 17) in which the activity of liver was so little in evidence as to lead us to suspect some error. It was found that the acid used in making up the stock adrenaline was *N* instead of

N/10. The activity of liver does not seem to be dependent on a simple catalysis of auto-oxidation, consequent upon contact of the tissues with metal in the course of their preparation. Even a bright copper strip or copper filings added to blood and adrenaline, or to blood, liver and adrenaline, does not affect appreciably the disappearance of adrenaline activity under conditions where neither the blood nor the liver has hitherto come into contact with metal. This is in direct contrast to what happens when metallic copper is added to a solution of adrenaline in Ringer's fluid, as first pointed out by Schild [1933]. The fact that the inactivating power of liver is abolished by boiling is also against the view that such an effect could be responsible.

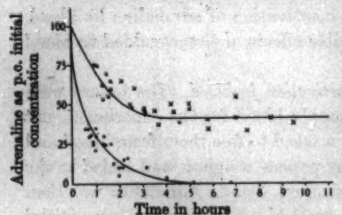

Fig. 2.

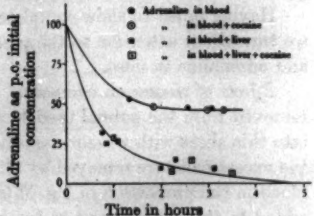

Fig. 3.

Fig. 2. To show contrast between inactivation of adrenaline in oxalated or defibrinated blood with and without addition of liver slices. Upper curve shows inactivation in blood alone: lower curve inactivation in blood to which liver (1 g./ml.) has been added. Initial concentration of adrenaline 20γ/ml. Data from Exps. A 30–34, 36, 37, 41–43, 51, 52, 56, 62, 67 and 68.

Fig. 3. To show that cocaine does not inhibit the inactivation of adrenaline in blood or in blood to which liver has been added. Initial concentration of adrenaline 20γ/ml. and of cocaine 10γ/ml. Data from Exps. A 61 and 62.

Liver ground with sand is active; but, when ground with sand in saline or Locke, filtrates from such concoctions, as free as possible from cells and from cell fragments, have proved inactive in our hands. Similarly, cell free extracts with *N*/100 HCl, with 25 p.c. glycerol and with acetone were inactive. Our results with extracts were thus disappointing [cf. Schütz, 1933; Blaschko *et al.* 1937] and we could only be sure of results when we used liver slices or the unfiltered concoctions got by grinding the material with sand. We preferred the slices.

Attempts to abolish the activity of liver by means other than heating were not satisfactory. We could diminish the activity considerably by withholding oxygen but we never succeeded in abolishing it completely

even by bubbling N_2 through the blood. Addition of KCN had no effect in the doses which we investigated (10–20γ/ml.) and formol, which we also tried, proved useless because of its own inactivating effect on adrenaline [Cramer, 1911], an effect quite evident with as little as 5γ/ml.

Effect of cocaine on adrenaline inactivation. Though the point was perhaps not yet proven, liver appeared to produce an effect on adrenaline which might provisionally, at least, be attributed to an enzyme (oxidase) and the last part of our first object, as explained in the introduction, was to determine whether cocaine had an inhibitory effect on this action and on the action of blood itself so far as this went. (For a review of the early literature relating to the action of cocaine on the effects of adrenaline and of sympathetic nerve stimulation see Trendelenburg [1924].)

In making up the experimental solutions cocaine hydrochloride was first added to a portion of the blood; liver slices were then added to a portion of the blood and cocaine, and to a portion of control blood; the adrenaline was added to control and to experimental fluids last of all. In some experiments the liver was added last of all. The particular procedure used made no difference to the results. The concentrations of cocaine used were from 5 to 20γ/ml., i.e. a total per ml. many times greater than that sufficient to produce a demonstrable effect on adrenaline sensitivity when injected intravenously into a cat.

In determining the adrenaline values of blood, etc., in which cocaine was present, it was found convenient to inure the test animal to the effects upon adrenaline sensitivity of further additions of cocaine by administering from 1 to 10 mg. of cocaine hydrochloride subcutaneously before the experiment started. Failure to do this resulted in highly inconsistent results due to the fleeting and/or step-like changes in adrenaline sensitivity induced by the minute amounts of cocaine in the experimental injections.

Fig. 3 illustrates the results of two such experiments. Cocaine, added to blood or to blood and liver, has no inhibiting effect on the inactivation of adrenaline under the conditions of our experiments, and thus, presumably, does not produce its well-known potentiating action on the effects of adrenaline, and on certain actions mediated by sympathetic nerves, by inhibiting adrenaline inactivation.

From the experiments so far described we see that the hypothetical analogy between the mechanisms responsible for the inactivation of adrenaline and of acetylcholine breaks down in one important respect, for while whole blood is extremely active in destroying acetylcholine this is not so with adrenaline. The hypothetical analogy between the actions

of eserine and cocaine, in so far as these substances affect the actions of acetylcholine and adrenaline and the actions of autonomic nerves, breaks down completely. Thus while eserine inactivates choline-esterase, cocaine fails to inactivate the inactivating principle (or principles) of tissues and so must produce its potentiating or "sensitizing" action to adrenaline and to adrenergic nerve stimulation in some other way.

Part II. Further observations on adrenaline inactivation in blood

That an "equilibrium", with respect to inactivation, is reached in blood was confirmed by many experiments. For any given initial concentration the equilibrium concentration varies somewhat from blood

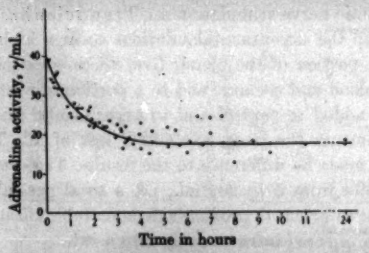

Fig. 4. To show variations of equilibrium value in different experiments with the same initial concentration of adrenaline (40γ/ml.). Data from Exps. A 47, 49, 51, 52, 53, 55, 57, 58, 63, 64, 67, 74 and 85.

to blood. This fact is evident from Fig. 4, which gives the graphed results of a number of experiments in which the initial concentration of adrenaline was 40γ/ml. In any individual experiment the equilibrium concentration is quite definitely established. The average value of the equilibrium concentrations for an initial concentration of 40γ/ml. was 17·8γ (13 experiments) and the individual values ranged from 14γ to 24γ/ml. (see Fig. 5*a*).

It is obvious that these individual variations may partly be due to errors and thus be insignificant; for if the initial concentration is more than that estimated by, say, 2γ/ml. and the standard solution used for assay less by 2γ/ml. than estimated, the assay (assuming there are no inherent errors in this) will give an equilibrium value 4γ/ml. higher than the actual; but the greatest care in measuring the blood and the adrenaline solutions has not diminished the variation, and we therefore consider it to be probably significant. As we shall see later, it may be related to differences in the corpuscle : plasma ratio in different samples of blood.

In addition to these individual variations for a given initial concentration there is a variation in the equilibrium concentration related to differences in the initial concentration of adrenaline. This relationship for different initial concentrations from 60γ downwards is shown in Fig. 5*a*. The points through which the graph is drawn represent the average values for that number of observations indicated for each initial concentration by the bracketed numerals on the right-hand side of the chart; while the small vertical lines, lateral to the main points, represent the range of the equilibrium values for each initial concentration. From

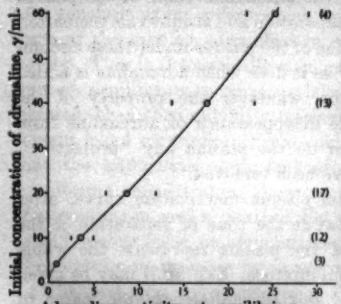

Fig. 5*a*.

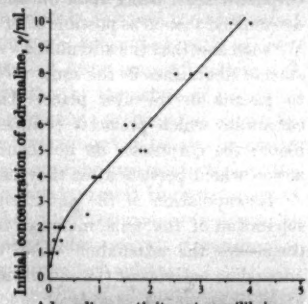

Fig. 5*b*.

Figs. 5*a* and 5*b*. To show variation in equilibrium value of blood and adrenaline with different initial concentrations. Fig. 5*a* shows the variation for initial concentrations from 60γ/ml. downwards. For description see text. Fig. 5*b* shows in greater detail the variations of the equilibrium value for initial concentrations from 10γ/ml. to 0·5γ/ml. Each point for a particular initial concentration on this chart represents a separate experiment. Data for Fig. 5*b* from Exps. A 8, 44, 64, 65, 66, 89 and 92.

a physiological point of view the most interesting part of this curve is that which relates to initial concentrations between, say, 10 and 0·5γ, and so that part of the curve is reproduced in greater detail as Fig. 5*b*. Unfortunately, however, it is with these small initial concentrations that our method tends to break down, for the simple reason that accurate estimates of the adrenaline activity at equilibrium involve the injection of relatively enormous quantities of blood (2–3 ml.). Nevertheless, the results, carefully controlled, are concordant so far as they go. But the experiments will have to be repeated using other techniques before we could feel justified in laying much stress on them. In the meantime

we can confine our attention to the results got with the relatively unphysiological initial concentrations of 20–40γ/ml. without prejudice as to what happens with very small initial concentrations.

The part played by the corpuscles in the inactivation of adrenaline by blood. The fact that adrenaline becomes completely inactivated in plasma and in serum but only partially in oxalated or defibrinated blood led us to consider the possible significance of the corpuscles in the phenomena exhibited by blood. We therefore made experiments in which oxalated blood, with added adrenaline, was allowed to come to equilibrium, the corpuscles then being spun off and the adrenaline value of the plasma determined as soon as possible after separation and at intervals thereafter. We soon saw that the adrenaline value of the plasma under these circumstances diminishes in the same way as it does when adrenaline is added to plasma in the first place. Thus, whatever the property of the corpuscles which prevents complete disappearance of adrenaline from blood, the corpuscles do not confer on the plasma any "protective" action which persists after they have been removed.

Extrapolation of the equilibrium plasma inactivation curve, after separation of the cells, indicates that at the time of separation of the corpuscles the adrenaline value of the plasma represents the whole adrenaline activity of the equilibrium mixture. Exp. A 57 may be given as an example: A sample of blood and adrenaline, 40γ/ml., came to equilibrium at 15γ/ml. Separation of cells from the plasma from 40 ml. of this equilibrium mixture gave 18 ml. of cells and 22 ml. of plasma. The adrenaline value of the plasma 30 min. after separation was 25γ/ml. and 2 hr. 50 min. after separation 18γ/ml. (average values for triplicate readings at 5 min. intervals). Extrapolation from these points, which lie in the linear part of the plasma inactivation time curve, gives the plasma adrenaline concentration at the time of separation as 26·5γ/ml. Now, if all the adrenaline of the equilibrium mixture (i.e. 15γ/ml.) is in fact present in the plasma, the adrenaline content of the separated plasma at the time of separation should be $(15/1 \times 40/22)\gamma$/ml., i.e. 27$\gamma$/ml. approximately, which is not significantly different from that found. Direct determination of the plasma or serum adrenaline immediately after separation confirms this type of result, and so we have the further important fact that all the adrenaline activity of an equilibrium mixture, as determined by bio-assay of the intact mixture, resides in the plasma or serum of the mixture.

It immediately appears, therefore, that the blood corpuscles play an essential part both in the establishment and in the maintenance of the equilibrium phenomena which we have described.

Adrenaline associated with the cells of an equilibrium mixture. Having shown that serum or plasma, freshly separated from an equilibrium mixture, appeared to contain an amount of adrenaline which accounted for the whole activity of the equilibrium mixture, it remained to confirm this by showing that the corpuscles from an equilibrium mixture showed by themselves no adrenaline activity. This was done. Whole corpuscles from an equilibrium mixture show no adrenaline activity when taken up in Locke solution and immediately injected into the test animal. If, however, the cells are left in Locke solution, or in fresh plasma or serum, the mixture soon acquires an adrenaline activity which gradually increases to a constant value, a fact which immediately suggests the survival of some of the missing adrenaline in reversible association with the corpuscles. This is dramatically demonstrated when corpuscles separated from an equilibrium mixture are laked either by the action of acid or by freezing: they then exhibit a marked adrenaline activity not shown by control laked corpuscles. From such observations it became apparent that the adrenaline equivalent of an equilibrium mixture, i.e. the equilibrium concentration, does not in fact represent the total amount of adrenaline in such a mixture, but that a further amount is present in or on the corpuscles, an amount incapable under ordinary circumstances of exhibiting its presence by an action on the test animal.

The next step was to determine the amount of adrenaline associated with the corpuscles of an equilibrium mixture. Two methods of doing this were open to us: One was to separate the cells from the mixture, determine the relative cell/plasma or cell/serum volumes, lake the cells and find their adrenaline value per unit volume; the other was to lake the equilibrium mixture with dilute hydrochloric acid and find the adrenaline value of the laked mixture. Both methods were used and some results are summarized in Tables I*a* and I*b*.

TABLE Ia. Recovery of adrenaline from equilibrium mixtures. Plasma adrenaline, from separated equilibrium plasma, is calculated as adrenaline equivalent per ml. original whole blood. Corpuscle adrenaline, from separated equilibrium cells, is calculated as adrenaline equivalent per ml. original whole blood

Initial conc. (γ/ml.)	Adrenaline value plasma (γ/ml. whole blood)	Adrenaline value cells (γ/ml. whole blood)	Adrenaline value cells + plasma (γ/ml.)	Total adrenaline recovered (p.c. of initial adrenaline)
20	9·4	5·9	15·3	76·5
40	14·8	14	28·8	72
40	15·8	12·4	28·2	70·5
60	26·2	19·1	45·3	75
			Average	73·5

TABLE Ib. Recovery of adrenaline from equilibrium mixtures. Corpuscle adrenaline by laking equilibrium mixture

Initial conc. (γ/ml.)	Equilibrium conc. (γ/ml.)	Adrenaline equivalent laked equilibrium mixture (γ/ml.)	Total adrenaline laked equilibrium mixture (p.c. original)
20	6·7	17·0	85
20	7·7	14·5	72
40	13·3	30·9	77
40	15·5	33·5	84
60	29·0	43·3	72
60	26·0	52·0	87
		Average	79·5

It will be seen that a somewhat greater "recovery" of adrenaline was got by laking the equilibrium mixture than by separating the cells from the serum or plasma. This difference may be accounted for by loss of cell-adrenaline in the experimental procedures necessary with the separation method. The average "recovery" or "total equilibrium" adrenaline from 10 equilibrium mixtures selected at random (initial concentration 40 and 20γ/ml.) represents approximately 80 p.c. of the original amount added. Thus while an average of 56 p.c. of the initial adrenaline (40 or 20γ/ml.) becomes physiologically inactivated by the time equilibrium is reached, all of this amount is not destroyed; a further 36 p.c. of the original amount can be recovered from the corpuscles in which situation it is ordinarily incapable of manifesting a pressor action on the test animal. We conclude therefore that the total irrecoverable adrenaline of an equilibrium mixture is actually only about 20 p.c. of the original, as against the apparent 56 p.c. of the original as given by the equilibrium adrenaline value.

If the procedure for obtaining an inactivation curve for blood and adrenaline is followed out and alternate samples of blood are laked immediately before assay two separate curves result, the normal ("apparent") inactivation curve, and what one might call for convenience the "virtual" inactivation curve. The uppermost graph in Fig. 6, constructed from the data of two experiments (A 63 and 64), is an example of such a "virtual" inactivation curve. It would seem from such results that the "irrecoverable" adrenaline is lost before equilibrium is reached, since the assay curve for the laked samples becomes horizontal before the normal inactivation curve does so.

Whether the irrecoverable adrenaline of an equilibrium mixture is destroyed by oxidation or otherwise, or is inactivated in the same way as the recoverable cell adrenaline but in a less easily reversible manner, or is inactivated by combination with some constituent of the blood,

irreversibly or otherwise, we are not at present in a position to say. Nor are we able to say whether the adrenaline recovered by laking an equilibrium mixture, or separated equilibrium corpuscles, is inactive because it is inside the corpuscles in a free state, or is adsorbed on the corpuscles, or both. Whatever be the truth it is certain that, so far as the recoverable adrenaline associated with the corpuscles is concerned, the mechanisms involved can be partially reversed under circumstances not involving destruction of the corpuscles. If, for example, cells from an equilibrium mixture are added to fresh serum or plasma this mixture acquires and shows a progressively increasing adrenaline value which ultimately becomes constant—that is, comes to equilibrium: the adrenaline value of the separated cells diminishes accordingly. Similarly, if fresh blood is added to an equilibrium mixture the equilibrium changes and in 3–5 hr. has a value near to that which it would have had if the initial adrenaline had been added to the final volume of blood in the first place, and the adrenaline value of the cells is diminished in consequence.

What is perhaps a more important point, and one which may be considered as giving evidence that the recoverable adrenaline associated with the cells is normally inactive simply because it is within them, or is adsorbed by them, and that the irrecoverable adrenaline may be lost in another way, is the fact that the *integrity* of the corpuscles does not appear to be necessary either for the loss of the irrecoverable adrenaline or for the persistence of that amount normally associated with the cells and normally recoverable after laking either the equilibrium mixture or the separated equilibrium cells. This is illustrated by the following experiment (A 85):

Oxalated blood, collected aseptically, was divided into two portions. One of these was laked.

In this experiment, and also in most of those in which we studied inactivation of low initial concentrations, the blood was laked by freezing with liquid air. 10 ml. or so of blood at a time were placed in a hard glass test-tube the bottom of which was dipped in liquid air until the blood froze solid. It was then rapidly thawed by running warm water over the outside of the tube. Often the whole process was repeated to ensure thorough laking.

Samples of each portion having been retained as controls adrenaline was added to each of the remaining portions to give an initial concentration of 40γ/ml. Experimental and control samples were then incubated with continuous mechanical agitation in stoppered sterile vessels. Immediate assay gave initial values of 39 and 41γ/ml. for the normal and the laked portions respectively. 24 hr. later the adrenaline value of the normal sample was 16·7γ/ml. and of the laked sample 29γ/ml. A portion

of the normal 24 hr. sample laked with liquid air had an adrenaline equivalent of 30γ/ml. The 24 hr. equilibrium value is thus well within the normal limits, as is the total recoverable adrenaline from this equilibrium mixture. The adrenaline value of the sample laked before the adrenaline was added to it 24 hr. previously is, however, not significantly different from the adrenaline value of the laked equilibrium mixture. It thus appears (1) that the irrecoverable adrenaline does not depend for its loss upon the integrity of the corpuscles, and (2) that the total recoverable adrenaline is the same whether the corpuscles are intact or not, that is, whether part of it is in a state in which it is incapable of exercising an effect on the test animal by reason of association with intact corpuscles, or is free in the plasma along with destroyed corpuscles, in which situation it is free to exercise its action on the test animal from the beginning.

Relative amounts of adrenaline associated with cells and with plasma or serum of equilibrium mixtures. A further interesting point relating to the corpuscle adrenaline is that at equilibrium the adrenaline equivalent of the corpuscles appears to be greater, volume for volume, than that of the plasma. If, for example, making use of the 4 : 6 corpuscle/plasma ratio determined by the hæmatocrite and the equilibrium and laked equilibrium values given in Table I*b*, we calculate the corpuscle adrenaline and plasma adrenaline concentrations respectively for each of the six blood samples of that table, we find the average values for each of the three sets of initial concentrations to be 21·4 and 12γ/ml. for the original 20γ/ml. samples, 44·5 and 24γ/ml. for the original 40γ/ml. samples, and 50·4 and 45·8γ/ml. for the original 60γ/ml. samples.

If we calculate the plasma and corpuscle adrenaline concentrations from the average equilibrium values for different initial concentrations (see Fig. 5*a*), assuming an 80 p.c. recovery and a 4 : 6 corpuscle/plasma ratio, the differences are not so striking as those just quoted; for an initial concentration of 10γ/ml. they are for corpuscles and plasma, in that order, 10·25 and 6·5γ/ml.; for 20γ, 18 and 14·7γ/ml.; for 40γ, 35·7 and 29·5γ/ml.; and for 60γ, 56·2 and 42·5γ/ml. respectively. The differences are, of course, reduced still further if the total recovery is reduced or the corpuscle/plasma ratio approaches nearer to unity. But that the corpuscle adrenaline concentration at equilibrium probably is significantly greater than the plasma adrenaline is confirmed by experiments in which the total recovery was accurately estimated and the corpuscle/plasma ratio determined at the outset by the addition of known volumes of spun cells to known and widely different volumes of separated plasma. In the

experiment which we quote, spun cells from oxalated blood were added to separated plasma in four different ratios; adrenaline was added to give an initial concentration of 20γ/ml. of the mixtures, and allowed to come to equilibrium. In addition to the equilibrium values, the total adrenaline recovery was determined in each instance; from these figures, together with the known corpuscle/plasma ratios, the plasma and corpuscle adrenaline concentrations were calculated. These results are set forth in Table II. (It must be clearly understood, however, that in speaking of

TABLE II. Equilibrium values, total recovery, and corpuscle and plasma adrenaline values in blood with altered corpuscle/plasma ratios. Initial concentration of adrenaline 20γ/ml. in all cases. Cell (or plasma) conc. $= \left(c \text{ [or } p\text{] amount} \times \frac{\text{total volume}}{c \text{ [or } p\text{] vol.}} \right)$

Ratio cells : plasma	Equilibrium value (i.e. plasma adrenaline) (γ/ml.)	Adrenaline equivalent laked equilibrium mixture (i.e. plasma and cell adrenaline) (γ/ml.)	Total adrenaline recovered (p.c. initial conc.)	Plasma adrenaline conc. (γ/ml. plasma)	Cell adrenaline conc. (γ/ml. cells)
4 : 2	3·9	15·3	76·5	11·7	17·1
4 : 6 (normal)	7·5	14·3	72·0	12·5	17·0
4 : 10	9·7	16·0	80·0	13·6	22·0
4 : 16	11·0	16·7	84·0	13·7	28·5

the "cell concentration" of adrenaline we do not infer that the adrenaline is in fact in simple solution within the cells at equilibrium: adsorption may well be the main, or indeed the exclusive factor at work. At the present time we cannot commit ourselves either one way or the other.)

In an earlier part of this paper it was pointed out that the individual variations in the equilibrium concentrations for different initial concentrations of adrenaline might be partly accounted for by differences in the corpuscle/plasma or corpuscle/serum ratios in the different samples of blood. The effects upon the equilibrium value of alterations in the corpuscle/plasma ratio, as in the experiment just quoted, show that this view is perhaps justified.

Experiments with low initial concentrations of adrenaline. We have hitherto dealt for the most part with results arrived at in the study of initial concentrations of 20 and 40γ of adrenaline per ml.; we revert finally to the question of much lower initial concentrations. We have already alluded to these (p. 243), and to the difficulties of investigating them by our method. The chief difficulty arises from the relatively enormous quantities of blood that have to be injected in the later stages of such experiments in order to get an estimate of the adrenaline present.

The number of injections is thus limited and the difficulties both of making an assay and of making adequate controls are further increased by this restriction. More serious, however, than the difficulties introduced by the simple volume factor is the fact that the controls often cause an atropine-resistant depressor effect which is greatly exaggerated when the blood is laked. Sometimes these presumably histamine effects are absent even with 5 ml. injections of blood: there is considerable variation either in the content of the depressor substance in different samples of blood and/or in the sensitivity of the test animals to it. In view of these difficulties, however, we regard our results with these low initial concentrations with caution until such time as the experiments can be repeated using other techniques.

Keeping this proviso in mind we present in Table III the results of an experiment (A 92) conducted under the best conditions, i.e. where the

TABLE III. To show equilibrium values, total recovery and relative corpuscle and plasma adrenaline concentrations with low initial concentrations. Figures in brackets are the percentages of the original concentration represented by the figures which precede them

Initial conc. (γ/ml.)	Equilibrium conc. (γ/ml.)	Adrenaline conc. laked equilibrium mixture (γ/ml.)	Cell conc. (γ/ml. cells)	Plasma conc. (γ/ml. plasma)
5·0	1·8 (36)	3·7 (74)	4·75	3·0
2·0	0·45 (22·5)	1·24 (62)	1·97	0·75
1·5	0·25 (16·6)	0·70 (50)	1·12	0·42
1·0	0·075 (7·5)	0·25 (25)	0·44	0·12
0·5	0·025 (5)	0·10 (20)	0·19	0·04

sensitivity of the test animal to adrenaline was extraordinarily high and the controls had no apparent histamine activity. The chief points of interest appear to be: (1) that as the initial concentration of adrenaline is lowered from 5 to 0·5γ/ml. the percentage of the original adrenaline represented by the equilibrium value falls from 36 to 5, and the total recovery from 74 to 20; (2) at equilibrium the "cell concentration", expressed as a multiple of the plasma concentration, rises from about 1·6 for an initial concentration of 5γ/ml. to about 4·7 at 0·5γ and this despite the fact that if histamine is interfering with the assays it would tend to diminish the observed cell concentration of adrenaline to a greater extent than the plasma concentration. If adsorption is the main factor at work in determining the phenomena which we have described in blood it may be that the comparatively low equilibrium values with the very small initial concentrations are due to more complete adsorption, and the low recoverable adrenaline to very incomplete reversal of the process, under these circumstances.

Concluding remarks

Detailed discussion of the results reported in this paper would be premature at this stage and must wait until some further facts are forthcoming. One or two observations of a general nature may, however, be permissible now. In the first place, we would point out the advantage of using blood as a medium in experiments on adrenaline inactivation. Despite the fact that Oliver & Schäfer in 1895 pointed out the feeble adrenaline inactivating power of blood, most studies of adrenaline inactivation since that time have been carried out using Ringer fluids as the medium. The rapidity of free oxidation in this medium, taken together with the rapid inactivation of adrenaline *in vivo*, appears to have led many, at least implicitly, to identify the processes in Ringer's fluid and *in vivo*, or if not going so far, at least to regard the process in Ringer's fluid as the standard against which inactivation by tissues or other factors is to be measured. This is seen, for example, in Miss Wiltshire's [1931] work. By taking blood as a medium and the inactivation of adrenaline in blood as a physiological standard the fallacious arguments and the contradictory interpretations which arise when other media are used would to a large extent be obviated.

The only general conclusion which we can safely draw from the work recorded here is that blood itself is probably but of small significance in determining the inactivation of adrenaline in the body and that it is in the tissues almost exclusively that the conditions for this inactivation are fulfilled. It is true that under the conditions of our experiments complete inactivation of 40γ adrenaline by 1 g. of liver—the most active of all the tissues we examined—takes some 4–5 hr. But this length of time does not in our view make it unlikely, as Bacq [1936] rather seems to suggest, that the same mechanism is in fact the one responsible for the rapid inactivation of adrenaline *in vivo*. In the first place, the conditions under which our experiments are carried out are manifestly much less favourable to the inactivating property of the tissues than are the conditions *in vivo*. In the second place, even if 1 g. of liver does take 4 hr. to inactivate 40γ of adrenaline, surely it is not too much to expect the 80 g. or so of cat liver *in vivo*, taken together with the lesser but very significant inactivating effect of many if not most of the other tissues of the body, to inactivate the same amount in a few minutes. Apart from such considerations, however, an examination of the inactivation-time curves for the different media we have investigated will immediately show how different are the inactivation *rates* in these media. Thus the

approximate times for 25 p.c. inactivation are in blood plasma 180 min., in serum 80 min., in blood 40 min., and in blood + liver 6 min. These facts are illustrated in Fig. 6 which summarizes most of our results. The relatively high initial rate of inactivation by liver is obvious from the graph, and the tailing-off may well be due partly to accumulation of oxidation products, a factor which might not operate to anything like

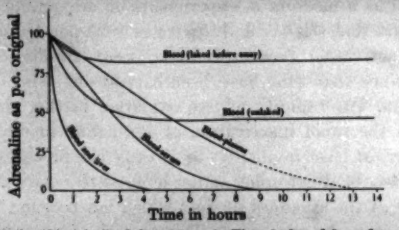

Fig. 6. To summarize the principal data given in Figs. 1, 2 and 3, and to show in addition the type of inactivation curve obtained by laking, immediately before assay, blood to which adrenaline has been added at zero time (see p. 246).

the same extent *in vivo*. But in a later paper one of us will revert to this topic and will show, among other things, that the activity of cat's liver in respect of the adrenaline inactivating principle is not nearly so great as is that of the liver of some other mammals, including man.

Summary

1. The methods used in studying the inactivation of adrenaline are described; all the experiments were made with cat's blood and cat's tissues and the adrenaline determinations made by blood pressure assay on spinal cats.

2. In oxalated blood plasma and in blood serum inactivation of adrenaline proceeds slowly to completion; it is more rapid in serum than in plasma.

3. In oxalated or defibrinated whole blood inactivation is never complete; it proceeds to what it is convenient to call an equilibrium value beyond which no further inactivation can be demonstrated.

4. Addition of tissue slices, or of concoctions of tissues, to blood and adrenaline leads to the complete disappearance of adrenaline activity from the blood. Many tissues show this activity to some extent but the most active, weight for weight, is liver.

5. The adrenaline inactivating power of liver is not diminished by cyanide or cocaine: it is diminished by increased acidity of the medium and by the withholding of oxygen; it is absent after the tissue has been boiled.

6. Further study of the inactivation of adrenaline by blood showed that the equilibrium concentration varies with the initial concentration and that there is also some variation, for a given initial concentration, from sample to sample of blood. These last variations are probably due to variations in the cell/plasma or cell/serum ratios.

7. The adrenaline value of an equilibrium mixture is represented exclusively by adrenaline in the plasma or serum, but a further amount of adrenaline is present in or on the cells of such a mixture, an amount which ordinarily is incapable of manifesting its presence by an action on the test animal.

8. This additional adrenaline is in reversible association with the cells and can be recovered in maximum amount by laking the separated cells or the equilibrium mixture, or, in lesser amount, by placing separated equilibrium cells in fresh plasma or serum or Locke solution. Over 80 p.c. of the original adrenaline (20–40γ) added to blood is recoverable by laking an equilibrium mixture.

9. The total adrenaline recoverable from blood seems to be independent of the integrity of the cells.

10. Whether the association of adrenaline with the cells is one of simple adsorption, or of passage of adrenaline within the cells, or both, is not clear. Nor is it clear whether the 20 p.c. of original adrenaline irrecoverable from such a laked equilibrium mixture is irrecoverable because it is destroyed, or is combined in some way with some constituent of the blood, or is held inactive in the same way as the recoverable cell adrenaline but in a manner which renders its association irreversible under the conditions of our experiments.

The expenses of this work have been partly met by a grant to one of us (W. A. B.) from the Government Grant Committee of the Royal Society.

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THE MECHANISM OF PITCH CHANGE IN THE VOICE

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THE object of the work described in this paper was to study the action of the laryngeal mechanism in sopranos. For this purpose radiographs were made of a mesial, sagittal section of the head from the level of the nasal cavity to the larynx, while the subject was singing the vowel [*a*]. An analysis of the sound wave-form was made simultaneously with both a cathode ray oscillograph and a sound-on-disc record of the voice. If required, a motion picture recorded the subject's facial expression and the dimensions of the oral cavity could be determined by palatographic methods.

METHOD

For this study the X-ray apparatus used was a 10 kVA. Muller tube mounted horizontally. The exposure was reduced to $\frac{1}{60}$ sec. at 150 mA. with 80 kV. Each exposure was made on one-half of an 8×10 in. film and the moment of exposure controlled by a technique devised by the author [1937]. By this means the exposure could be made at any desired interval after the subject had attained the desired pitch, volume and quality. The subject's head was 12·8 cm. in width and the target was placed at 64 cm. distance from the tip of the nose. Hence the conical enlargement of the image was kept to 10 p.c. The cathode-ray oscillograph recorded on standard orthochromatic cine film run at 2 m./sec. with a time mark of 600 cycles. The microphone and amplifier for the oscillograph had an overall frequency response of ± 2 db. from 100 to 8000 cycles. A synchronized sound-on-disc record was made of each study. The subject was chosen as typical of a group of six soprano voices which were studied. She was of American parentage, had had 3 years' training and had a good soprano voice covering the range from 208 to

1024 cycles. She was of small build and stature and had a small larynx. During the study she was seated in order to keep the cheek against the vertical casette, but no head-fixing harness was used. Every effort was made to put her at ease and she was apparently little disturbed by the method. She sang with a narrow jaw opening and the volume was not loud. After the study the acoustic record was played to a jury of observers who gave an analysis of the voice and vowel qualities. The illustrations were made from contact prints of the negatives and for reproduction it was necessary to outline the important lines of the tongue, palate, pharynx, etc. The median line is always indicated as a solid line while the outlines of recessed parts such as the edges of the tongue are indicated by dotted lines. On each figure the oscillogram is mounted in the top, left corner, with the phonetic analysis of the sound and the pitch of the voice below. Below that again the index letter of the subject and the number of the radiograph are given. The cervical vertebræ are identified and the distance between the right edge of the oscillogram and the mark on the upper lip represents 10 cm. measured along the median line of the singer's body.

Results

The jury commented on the acoustic record as follows. In Fig. 1 the subject sang down a third and the voice was weak and throaty. In Fig. 2 the voice was still hoarse but the volume increased. In Figs. 3, 4, 5 and 6 the voice was a good soprano and the volume was good. In Fig. 7 the voice was shrill with a slight vibrato and the vowel quality was weakened. In Fig. 8 the voice was very shrill with little characteristic vowel nature. In the table the cavity dimensions and changes in position of the speech organs are analysed. All the measurements are relative to the positions shown in Fig. 1, and represent closely the actual dimensions on the singer's body.

The effect of rising pitch on the width of the lip opening and jaw aperture is very noticeable. This may result from the technique of voice training, despite the fact that the subject sang with a close mouth opening. It may result also from increased tension in the genio-hyoid muscle, which elevates the hyoid but lowers the mandible also. Tension in this muscle may be sensed by touching the underside of the chin during the phonation of high-pitched sounds. The total movement of the head is small and for this reason the changes in position of the hyoid bone and longitudinal cartilages are even more noticeable. The lip opening corresponds closely to the jaw opening and there is no protrusion of the lips from sphincteric action of the lip muscles. Throughout the study there is

no indication of opening by the velar passage into the nasal cavity, though, as shown in Fig. 8, the uvula may change position during the course of the very rapid exposure. The changes in shape of the uvula may indicate tension and relaxation. The sound records gave no indication of nasality for any of the vowels. The tongue positions for the vowel remain relatively constant. Transverse concavity of the tongue appears characteristic of singers and may be produced by contraction of the styloglossus muscle which retracts the tongue and elevates the sides. Transverse concavity of the anterior dorsum occurs when the tongue is retracted in the mouth.

For the vowel in question it seems that such a humping-back of the tongue in the mouth and narrowing of the pharyngeal cavity are characteristic factors. This fact is inconsistent with the theory of wide pharyngeal opening in singing. Other radiographs made by the author show that a wide pharyngeal cavity is not strictly necessary for the production of loud tones, and in one case the subject produced a powerful tone with a cavity opening of about 4 mm. In the present study the subject tended to keep the tip of the tongue behind the lower teeth in accordance with the technique of voice training. The aperture at the laryngeal vestibule was never more than 4 mm. and often as little as 2 mm. It is interesting that the narrowest such opening occurred on the lowest and highest pitches, thus confirming the findings of laryngo-periscopic studies. The present series of radiographs shows that such laryngo-periscopic studies are possible only when the epiglottis can be kept up out of the way. Accordingly, it is obvious that the X-rays give information about the physiology of human speech in a way that would be impossible by other means. Throughout the study the most noticeable fact was the upward movement of the hyoid bone and the longitudinal cartilages relative to the cervical vertebræ as the pitch was raised. Furthermore, for the highest pitches the larynx was drawn up closer to the hyoid bone. The upward movement of the hyoid bone was accompanied by a forward movement indicating contraction of the genio-hyoid, the mylo-hyoid and the digastric muscles. The measurements in the table on p. 258 indicate that this forward movement of the hyoid bone is partly checked by the antagonistic pull of the laryngeal muscles under tension. In Figs. 3, 4 and 5 the forward movement was checked and the hyoid bone did not change position relative to the lower jaw. This progressive upward movement of the larynx must involve increasing tension in the internal laryngeal muscles, including the vocal cords. This increasing tension may result in the narrowing of the larynx ventricle vertically and in a closer approximation of the ventricular bands

Fig. 1.

Fig. 2.

To face p. 356

Fig. 3.

Fig. 4.

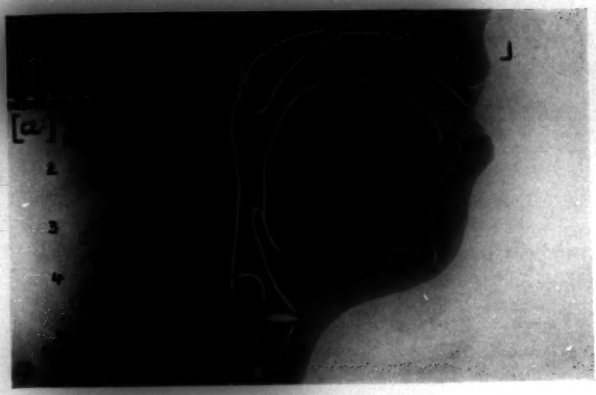

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

to the vocal cords. It would appear, however, that the mechanism of pitch change in the voice involves not merely tension of the vocal cords but also bodily upward movement of the whole larynx.

Summary

A series of eight radiographs, depicting a mesial, sagittal section of the head from the nasal cavity to the larynx, is presented for the study of pitch change in a soprano voice singing the vowel [*a*] over the range from 208 to 1024 cycles. Simultaneous cathode-ray oscillograms and sound-on-disc records were made. The dimensions of the speech cavities and positions of the speech organs are given in tabular form on p. 258 and the following results are demonstrated.

1. The jaw and lip opening increases with rising pitch presumably as a result of voice training.

2. The position of the tongue is characteristic of the vowel.

3. The pharynx opening and aperture at the larynx vestibule are consistently narrow and the narrowest larynx openings occur on the lowest and highest pitches.

4. A progressive upward movement of the hyoid bone and longitudinal cartilages corresponds to the rising pitch. This upward movement amounts to 18 mm. over the range of more than two octaves.

5. The rising pitch results in a narrowing of the larynx ventricle vertically and a closer approximation of the ventricular bands to the vocal cords.

The work was carried out during the author's tenure of a Commonwealth Fund Fellowship in Speech at Ohio State University, U.S.A. The author would like to acknowledge his great indebtedness to Prof. V. A. Ketcham, Prof. G. Oscar Russell and to Dr J. C. Cotton for encouragement and direction, to Dean McPherson and the Graduate Council for the provision of apparatus, and to teachers and students of the Department of Music who gave valuable information and acted as subjects for this study.

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DESCRIPTION OF PLATES I–IV

Figures	1	2	3	4	5	6	7	8
Vowel	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
Pitch in cycles/sec.	208	384	460	566	630	760	825	1024
Jaw opening in mm.	9	10	10	9	9	13	15	23
Lip opening in mm.	10	12	15	13	15	17	20	30
Pharynx opening in mm.	6	7	8	7	9	10	8	8
Head tilt to spine in degrees	60	65	62	65	62	60	62	62
Upward movement of hyoid in mm.	0	8	9	10	13	13	15	16
Forward movement of hyoid in mm.	0	4	6	6	7	7	9	10
Aperture of the larynx in mm.	2	3	3	4	3	3	2	2
Upward movement of larynx in mm.	0	5	8	9	13	13	15	18
Concavity of tongue in mm.	1	3	2	2	2	2	3	3
Oral cavity opening in mm.	22	22	21	21	23	23	23	27
Movement of lower jaw in mm.	0	3	2	3	3	3	2	2

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THE EFFECT OF ANAEROBIC ACTIVITY AND REST ON THE EFFICIENCY OF CONTRACTION IN ISOLATED FROG MUSCLE

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Numerous studies have confirmed the general proposition that in a muscle twitch the heat produced is proportional to the tension. Thus, the ratio of the tension (T) to the initial heat (H) when multiplied by the muscle length was found by Hill to have a value of 6·14 for various muscles under a variety of conditions. This appeared to indicate a fundamental regularity in the conversion of chemical to mechanical energy, and indicated that a constant proportion of the total energy mobilized passed through the stage of elastic potential energy. However, it has long been known that in advanced fatigue the efficiency declines, and Hill [1931] has made some observations indicating an increase in the efficiency of the twitch of the isolated gastrocnemius muscle in oxygen during experiments in which the muscle was subjected to periods of tetanic stimulation.

Recent investigations in the field of muscle chemistry have revealed a number of previously unknown reactions associated with contraction. The great complexity of the chemical mechanisms now believed to be involved gives ample latitude for the explanation of changes in efficiency. In view of this newer knowledge of the chemical background of muscle energetics and the more exact methods now available for the quantitative determination of heat and tension, it was decided to follow closely changes in T/H related to environmental factors and to the extent of functional activity. In the present communication we describe changes in the efficiency of contraction occurring in resting and active muscles during prolonged periods of oxygen lack.

Methods

The essential requirements are methods for the accurate measurement of the initial heat and the tension associated with a twitch. For the heat production the standard procedure, involving the use of a sensitive thermopile-galvanometer system, developed by Hill [1928] and previously used in this laboratory, was utilized. The sensitivity of the system was such that the deflexion due to the warming of the muscle following a single twitch amounted to from 150 to 300 mm. on a scale placed 3 m. from the galvanometer mirror. The maximum deflexion was used as a measure of the initial heat production. With a properly prepared muscle, successive readings showed a variation well under 1 p.c.

The tension was obtained through a photographically recording isometric lever, which gave a tracing between 60 and 120 mm. in height for the twitch tension in the fresh muscle. The large deflexions were obtained by placing the camera several metres from the myograph mirror, so that there was no sacrifice of the rigidity of the set-up. The tensions could then be measured with exactness and, at the same time, very little shortening was permitted. The smoothness of the curves, examples of which are reproduced in the figures, testifies to the uniformity and exactness of the individual determination of T/H made under the conditions of these experiments.

All the measurements were related to the initial values in the fresh muscle, which made it unnecessary to convert the tension and galvanometer deflexions into absolute units of energy. Since the length of the muscle was constant for each experiment, it also could be neglected. The efficiency (T/H) of each twitch was calculated as a percentage of that of the first few twitches after the start of the experiment. In all the experiments a double sartorius preparation from *Rana pipiens* was employed. The muscles were subjected to a preliminary period of soaking in buffered Ringer's solution (NaCl 0·675 p.c., $CaCl_2$ 0·020 p.c., KCl 0·015 p.c., phosphates 0·010 p.c., to give a *p*H reading of 7·2), through which oxygen was slowly passed. After about 2 hours the solution was removed and the oxygen replaced by nitrogen. At the end of another $\frac{1}{2}$ hour, measurements of the tension and the heat in single twitches were started. Stimulation was by single maximal break shocks through an induction coil or by the discharge of an appropriate condenser. Observations were generally made at intervals of from 3 to 5 min. over a period of several hours.

Results

During prolonged exposure to an oxygen-free atmosphere there was, after a time, a gradual falling off in the tension of the twitch. The heat production also became less, but not as rapidly as the tension, and efficiency thus fell. The results of some preliminary observations, in which the tension was recorded by the comparatively inaccurate method of kymograph tracings, are shown in Table I. With one exception it will

Table I.* Changes in efficiency (T/H) in relation to the fall in twitch tension in muscles resting in nitrogen

Exp.	Previous twitches	Time in hours	Tension p.c.	T/H p.c.
1/12/34	105	—	25	79
1/19/34	100	2½	25	60
1/22/34	115	3	20	60
1/24/34	45	2	20	50
1/29/34	20	2	20	100

* In this and subsequent tables the values for tension and T/H are expressed as a percentage of the value obtained at the start of each experiment.

be noted that, in the course of an experiment lasting several hours, when the tension of the twitch had fallen to from 20 to 25 p.c. of its initial value, the ratio of tension to heat was significantly less. This trend is more accurately shown in Table II where the figures for efficiency are

Table II. The effect of anaerobic "rest" on the tension-heat ratio in single twitches

Exp.	Stimulation interval	T/H ratios at various tensions (p.c.) 80	60	40	20
2/9/34	—	78 (13)	60 (18)	45 (23)	36 (32)
2/12/34	—	97 (10)	88 (23)	76 (29)	—
2/14/34	3 min.	92 (50)	73 (75)	55 (100)	43 (125)
2/16/34	3 min.	82 (136)	—	63 (160)	48 (180)
3/8/34	3 min.	92 (15)	81 (22)	66 (140)	—
10/25/35	1 hr.	80 (34)	60 (63)	53 (70)	—
10/29/35	3 min.	91 (18)	75 (30)	—	—
2/16/37	5 min.	100 (15)	89 (50)	68 (60)	48 (68)
	Average	89	75	61	44

shown for several points on the declining tension curve. In this and Table III the figures in parentheses denote the total number of twitches to which the muscle had been subjected prior to the observation. In this series of experiments the tensions were photographically recorded, and the results are consistent in showing a decrease of efficiency corresponding approximately to the fall in tension occurring during prolonged exposure to an anaerobic environment. Thus in the average of seven experiments

TABLE III. Tension-heat ratio of "exercised" muscles in nitrogen

Exp.	Duration in hours	T/H ratios at various tensions (p.c.) 80	60	40	20
2/19/34	2½	128 (210)	135 (262)	122 (365)	138 (470)
2/23/34	1½	105 (115)	105 (165)	102 (215)	95 (315)
2/26/34	1½	108 (50)	110 (100)	108 (175)	—
3/2/34	1½	94 (115)	103 (165)	100 (270)	106 (300)
3/5/34	1½	106 (60)	102 (70)	96 (80)	115 (140)
10/3/34	½	100 (60)	113 (120)	117 (200)	—
1/8/35	1	94 (120)	—	—	—
1/10/35	2½	100 (75)	109 (185)	—	—
1/15/36	1½	110 (50)	115 (165)	—	—
	Average	105	111	108	—

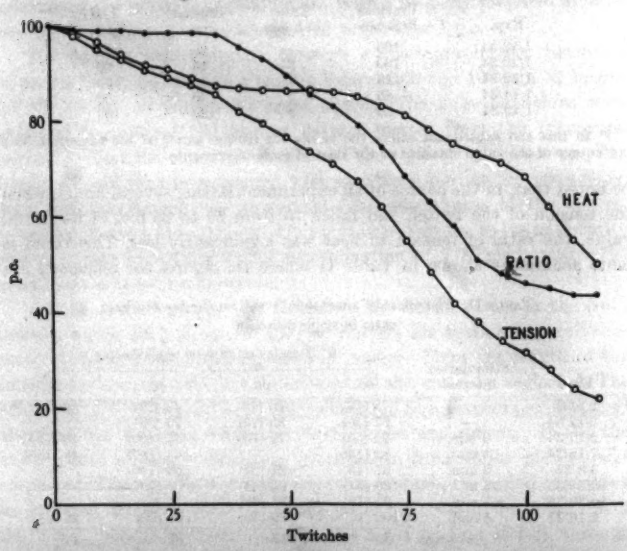

Fig. 1. Sartorius muscle "resting" in nitrogen. The curves show the changes in tension, heat and the calculated values of T/H over a period of about 6 hours. Each point represents the average result from five successive twitches obtained at intervals of from 3 to 4 min. throughout the period.

tabulated, the ratio of tension to heat at the point where the tension had fallen to 40 p.c. was only 61 p.c. of that at the start. A characteristic result is charted in Fig. 1 which shows the changes occurring in a muscle in nitrogen over a period of more than 6 hours.

In certain of the experiments of this type the attempt was made to speed up the rate of tension reduction by interposing a short tetanus or a number of rapidly repeated single responses. This led to the discovery that a muscle which was subjected to such extra activity subsequently showed an entirely different relationship between the changes in tension and efficiency. A series of experiments was then carried out in which the muscle was kept relatively active by the interpolation of about fifty twitches every $\frac{1}{2}$ hour or so during the experiment, the conditions otherwise being the same as before. The results are summarized in

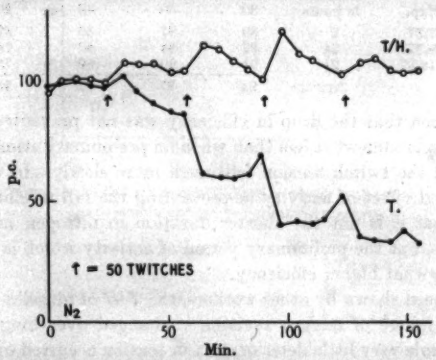

Fig. 2. The relationship between the tension (T) and muscle efficiency (T/H) in a muscle kept in nitrogen but subjected to extra twitches, as indicated by the arrows. Each point represents tension or T/H of a single twitch.

Table III, which gives the T/H ratios as percentages of the initial values at various tensions from 80 to 20 p.c. of the maximum obtained at the start. It will be seen that, by this procedure, the characteristic fall in efficiency is entirely prevented, and there is, in fact, an actual improvement as the tension of the twitch falls to a low value in the course of an experiment. A chart showing details of one experiment is given in Fig. 2. It will be noted that the T/H ratio is regularly raised immediately following the extra activity resulting from a series of single stimuli, and by the frequent repetition of such activity the efficiency is maintained at a high level. This was characteristic of all experiments.

In the last group of experiments, because of the extra activity to which the muscles were subjected, the reduced tension values were more quickly reached than was the case in the muscles of the "resting" group.

It was therefore possible that the time in nitrogen, apart from exercise, might have played a part in the difference. Additional experiments were therefore carried out in which the tension drop was speeded up by a preliminary period of stimulation (from 50 to 100 twitches or several short tetanic stimuli), the muscle thereafter being studied as in the "resting" series. The results of three experiments are given in Table IV.

TABLE IV. Tension-heat ratio of "resting" muscles after a preliminary period of stimulation

		T/H ratios at various tensions (p.c.)			
Exp.	Duration in hours	80	60	40	20
2/3/37	2	89	87	86	82
2/9/37	2½	92	84	80	78
2/18/37	2½	91	90	80	75
	Average	91	87	82	78

It will be seen that the drop in efficiency was not prevented, although the change was somewhat less than when no preliminary stimulation was applied and the twitch tension fell much more slowly. In view of the demonstrated effect of activity in preventing the fall in efficiency, it is probable that it is not the shorter duration in nitrogen in the latter experiments, but the preliminary period of activity which is responsible for the somewhat higher efficiency.

As has been shown by other workers, the T/H of muscles maintained in an atmosphere of oxygen remains unchanged over long periods of time. As a rule very little deterioration in tension occurred over a period of several days. In general, when the tension finally fell off, there was an accompanying slight fall in efficiency, but this was not always the case and was never as great as in the muscles maintained in nitrogen. In our preliminary note [1934] we described a transitory drop in T/H following activity of muscles in oxygen, such as is shown in the experiment charted in Fig. 3. Further experiments have shown that this is not constant, but that in many preparations activity causes the opposite effect, i.e. a transitory rise in T/H such as occurs under anaerobic conditions.

It is not uncommon to observe some reduction in T/H in muscles maintained in oxygen over a long period. An interesting illustration of this is shown in Fig. 4. This figure shows the heat and tension from a pair of sartorius muscles used in an experiment the previous day. The following morning the preparation gave extremely uniform responses with regard to both tension and heat during 1½ hours. It was then left at rest for 5 hours, after which the heat production in a twitch was uniformly greater and the tension less than had been the case before.

Some change had evidently occurred in the muscle which prevented the effective utilization of the chemical energy liberated.

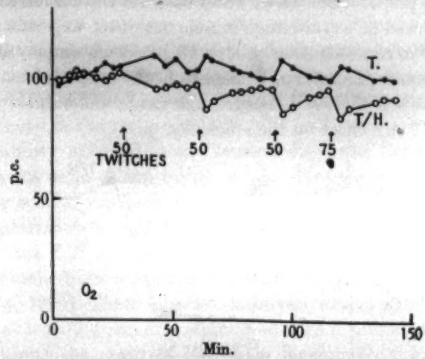

Fig. 3. Tension (T) and efficiency (T/H) of a muscle kept active in oxygen. Extra stimuli applied as indicated by arrows.

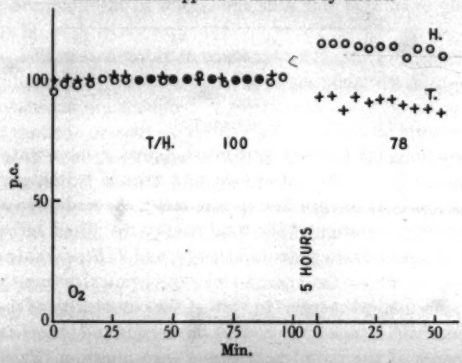

Fig. 4. Muscle at rest in oxygen shows spontaneous change in efficiency (T/H) due to increase in heat (H) and decrease in tension (T).

For reasons which are discussed later it was decided to determine whether changes in T/H could be brought about by changes in the pH resulting from exposure to carbon dioxide mixtures. The first experiment performed (using 25 p.c. CO_2 in O_2) gave a striking result and is reproduced in Fig. 5. It will be seen that this treatment caused a very large

increase in the heat production without a corresponding change in the tension of the twitch. The effect was reversible and was repeated several times in this preparation. In no other case did the carbon dioxide result in such a marked effect, although in nine out of ten experiments in which carbon dioxide concentrations above 15 p.c. were employed, there was some reduction in the ratio of tension to heat—varying from 5 to 25 p.c. When the carbon dioxide exposure was made anaerobically (CO_2+N_2) it had little if any effect on the efficiency.

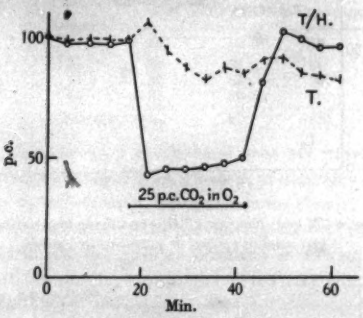

Fig. 5. The effect of carbon dioxide on the efficiency (T/H) of a muscle in oxygen.

Discussion

The experimental findings presented above demonstrate that the ratio of the tension to the initial heat in a muscle twitch may undergo marked changes in an oxygen-free environment. According to our present conception, only a fraction of the total energy mobilized actually passes through the stage of elastic potential energy, and T/H probably represents the efficiency at which the energy-yielding chemical processes are converted into mechanical energy. In view of the complexity of the chemical reactions involved, some of which are endothermic and others exothermic, the significance of the initial heat comes into question. This point has been discussed elsewhere [Cattell, 1936], and here it is sufficient to mention that the available evidence supports the view that the initial heat represents a measure of chemical energy set free in connexion with the process of contraction, to the exclusion of the secondary recovery mechanisms.

A second point of interest brought out by these experiments is the absence of the usual fall in efficiency when the muscle, in an oxygen-free

environment, was maintained in an active state by frequent stimulation. In some of these experiments the tension drop was brought about more rapidly than in muscles maintained in a condition of anaerobic rest, and it is possible that this may have played a small part in the result. That the shorter duration of anaerobiosis is not the main cause of the high efficiency of the active muscles is shown by the fact that the observations were continued for at least 2 hours, a period sufficient to cause a significant drop in efficiency in the resting muscle.

The possibility exists that the tension drop was the result of the failure to excite some fibres owing to the development of a state of inexcitability such as has been described by Dulière & Horton [1929]. In such circumstances a relatively high efficiency might be expected, for it is known that T/H is higher when only a small fraction of the total number of muscle fibres is stimulated [Hartree & Hill, 1921; Cattell & Brodman, 1930]. These muscles, however, were soaked in Ringer's solution for at least 2 hours and stimulated with maximal shocks, under which conditions the state of reversible inexcitability has not been observed. Further, the changes in efficiency are too large to be accounted for by the dropping out of fibres, but that this may have played some part cannot be completely excluded.

There is evidence, notably in connexion with the formation of hexosmonophosphate [Fisher & Cori, 1934], indicating that the basic chemical reactions are modified in relation to activity. It is from this standpoint that the present experiments have a special interest. If there should be a shift in the nature of the underlying chemical processes, it might be associated with a change in the fraction of energy passing through the stage of elastic potential. It has been suggested by von Muralt [1935], with reference to our preliminary report [1934] and his own experiments, that a certain amount of activity may be necessary in order to start the resynthesis of phosphocreatin, the phosphocreatin stores being thus, in the absence of activity, gradually depleted. The differences in chemical composition between anaerobically "resting" and "active" muscles are now being investigated.

It was thought possible that changes in *p*H might contribute to the fall in T/H in the "resting" muscle. Since in an anaerobic environment there is a gradual accumulation of lactic acid, this might bear a causal relationship to the lowered efficiency. The hydrolytic products of organic phosphate metabolism result in a less acid medium and, since the process is reversible, it might explain the temporary effects of activity. This hypothesis has received some support in the experiments described above

in which the muscles were exposed to an increased tension of carbon dioxide—a procedure which generally caused a reduction in T/H. It is well known that the viscosity of protoplasm is increased by exposure to acids, and that such treatment slows the twitch. These points suggest that the observed changes in efficiency might be mediated through influences affecting the viscosity. However, further work is required to determine to what extent the pH varies under the conditions of the present experiments. The method used by Stella [1929], i.e. the heat of reaction of carbon dioxide with the muscle buffers, has been used in some preliminary studies but it has failed to show an appreciable fall in combining power during periods of rest or moderate activity in nitrogen.

Summary

In a muscle maintained in an atmosphere of nitrogen at relative rest there was a gradual drop in the efficiency of the process of conversion of chemical to mechanical energy (elastic potential) as indicated by a fall in the ratio of the tension to the initial heat in the twitch response. In such an experiment the fall in T/H roughly parallels the drop in tension and finally reaches a very low value. In experiments in which the muscle was maintained in a state of activity by the occasional application of a brief tetanic stimulus or a series of single shocks, this drop in efficiency no longer occurred. Thus, in a muscle kept sufficiently active, a twitch may be accomplished with normal efficiency even after the tension has fallen to a small fraction of its initial value. Possible mechanisms are discussed.

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J. Physiol. (1937) 91, 269–278 612.111.2:612.621.5

THE RED CELL COUNT OF MACAQUES IN RELATION TO THE MENSTRUAL CYCLE

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Variations which occur in the daily water balance of the female pig-tailed monkey (*Macaca nemestrina*) during the menstrual cycle are related to cyclical changes in the size of the sexual skin, the swelling of which is mainly dependent on the subcutaneous deposition of fluid [Krohn & Zuckerman, 1937]. Although sexual-skin œdema can be accounted for by an increased retention of exogenous water, it was necessary to enquire whether or not water is transferred to the swelling skin from other tissues of the body (e.g. the blood). A second possibility was that the concentration of the red cells would alter if water were returned to the blood by the sexual skin during its phase of subsidence more rapidly than the excess water could be excreted by the kidney. The data presented in this paper show that the concentration of the blood in this species does in fact vary considerably and in a regular way during the menstrual cycle, and that dehydration and hydration of the blood are thus either directly or indirectly related to the sexual-skin phenomenon.

Similar fluctuations in the concentration of the blood in relation to the menstrual cycle have been stated to occur in Man, a species in which there is no sexual skin. As most of the reports on this matter are conflicting [see Krohn & Zuckerman, 1937] the question of cyclical variations in the blood count was studied on the rhesus monkey (*M. mulatta*), an animal in which cyclical activity of the sexual skin consists mainly in changes in coloration, and only very slightly in changes in size.

The investigation was extended in order to find whether or not any obvious fluctuation occurs in the daily water balance of the rhesus monkey, as in the pig-tailed macaque. Furthermore, since both the uterine cycle and the sexual skin in Primates are controlled by œstrin,

the influence of this hormone on the blood count and on the daily excretion of urine was studied in spayed rhesus monkeys which were being injected daily with it.

Technique

The hair on one of the arms of each animal was closely cropped or shaved, and the arm cleansed with tincture of iodine or spirit. Blood was obtained by stabbing with a lancet through the skin into the deltoid muscle. The first few drops were wiped away, and the sample was then pipetted according to the usual technique [Hutchison & Hunter, 1935]. All samples were taken within an hour of midday, and counted in a Bürker hæmocytometer. Each animal was tested twice weekly at as regular intervals as possible, the left and right arms being used alternately. At no time was blood taken from the same limb as had been used for any injection during the experiment under observation.

The ovariectomized rhesus monkeys used in this study were injected daily with oil solutions of different strengths of œstrone or œstrone benzoate. One animal received an oil solution of testosterone propionate. Œstrone was given for periods of 14 days, after which the animal was left uninjected till uterine bleeding occurred. New courses of injections were always begun on the second day of uterine bleeding.

The technique followed in the estimation of water balance is described below.

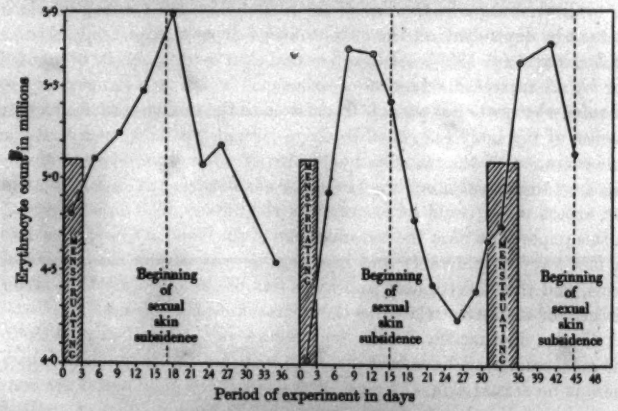
Erythrocyte count in millions
5·9
5·5
5·0
4·5
4·0
Beginning of sexual skin subsidence
Beginning of sexual skin subsidence
Beginning of sexual skin subsidence
0 3 6 9 12 15 18 21 24 27 30 33 36 0 3 6 9 12 15 18 21 24 27 30 36 39 42 45 48
Period of experiment in days

Fig. 1. The r.b.c. count during the menstrual cycle of the pig-tailed macaque, *Macaca nemestrina*.

Results

I. *Concentration of the blood in relation to œstrogenic stimulation*

(*a*) *The erythrocyte count in relation to normal sexual skin swelling* (Macaca nemestrina). The subject of this study was the pig-tailed macaque that had been used in the investigation of the relation of the menstrual

cycle to water metabolism. Observations of the red cell count were made during two complete cycles and the follicular phase of a third.

The concentration of r.b.c.'s increased during the follicular phase of each of the three menstrual cycles (when the sexual skin was expanding), and fell rapidly when the skin subsided (Fig. 1). There is considerable variation between the minimum counts observed in each cycle, but this variation does not affect the main change. The maxima are constant within the limits of experimental error.

(*b*) *The erythrocyte count in relation to the menstrual cycle in species where no sexual-skin swelling occurs* (Macaca mulatta). At the beginning of the investigation a few counts were made on normal rhesus monkeys (*M. mulatta*) at isolated points in the menstrual cycle. The results have been noted in our preliminary communication [Guthkelch & Zuckerman, 1937]. Two normal rhesus macaques (128 and 203) have since been studied more closely, the first for one cycle, and the second for three.

The r.b.c. count of 128 rose rapidly during the first half (follicular phase) of the menstrual cycle (from 5·18 to 5·73 millions) and then fell to a low level (4·59), to rise slightly before menstruation. The second animal (203) had a somewhat irregular menstrual history. The first cycle studied was 16 days long, the second 20 and the third 27 days. The concentration of red cells increased during the first 11 days of the first cycle (from 5·38 to 5·94 millions) and then fell rapidly, until the day before the onset of menstruation, to 5·23 millions. The count rose in the first half of the second cycle (4·77 to 6·14), fell in the second half (to 4·53), and increased significantly immediately before the onset of menstrual bleeding. It varied irregularly during the period of menstruation at the beginning of the third cycle, towards the end of the first half of which it increased (up to 5·65 millions), after which it decreased rapidly.

These observations show that the red cell count in the rhesus monkey fluctuates in the same way as in the pig-tailed macaque.

(*c*) *The erythrocyte count in relation to œstrin injections in ovariectomized rhesus monkeys.* Observations of the blood count were made regularly during, and after, nine courses of œstrin injections in five ovariectomized animals (see Table I).

In almost every instance the r.b.c. count increased during the course of injections and then fell gradually in the latent period that intervened between the cessation of injections and the onset of uterine bleeding. A second increase in the number of red cells was observed before uterine bleeding in six of the nine experiments.

TABLE I. Variations in r.b.c. count (in millions) of spayed female rhesus monkeys injected with œstrone or œstrone benzoate for 14 days

Exp.	First week of injections		Second week of injections		Min. count in latent interval before bleeding	Count at start of bleeding
	Min. count	Max. count	Min. count	Max. count		
69·1	4·86	5·25	5·06	5·39	4·77	5·38
69·2	5·14	5·14	5·44	6·02	5·18	5·31
71·1	4·74	4·76	4·80	5·58	4·59	4·85
71·2	5·47	5·47	5·40	5·50	4·80	4·93
74·1	4·59	5·55	5·41	5·89	4·67	4·37
74·2	4·90	6·26	5·60	5·81	4·46	5·07
75·1	4·83	5·04	5·23	5·45	4·96	4·94
186·1	4·85	5·26	5·04	5·41	5·14	4·69
186·2	4·32	4·69	5·42	5·98	4·88	5·26

These observations show that the blood becomes concentrated during phases of œstrogenic stimulation in the rhesus monkey.

(*d*) *The erythrocyte count during phases of bleeding.* Daily observations of the r.b.c. count were made throughout the phase of uterine bleeding in a group of six monkeys. Only three showed a decrease in the concentration of red cells during the period of observation.

(*e*) *The erythrocyte count in relation to the suppression of menstrual bleeding in a normal mature rhesus monkey.* Observations were made on a normal rhesus monkey (77) that was given injections of 25 mg. testosterone propionate twice a week, as a result of which uterine bleeding was inhibited. Injections were begun on the first day of a menstrual cycle, and continued for 6 months. Observations of the r.b.c. count began at the start of injections, and were made twice weekly for 9 weeks. The concentration of the blood was highest on the 11th and between the 46th and 50th days of the experiment, and lowest on the 32nd and between the 53rd and 60th days. It would thus seem that in spite of the suppression of menstrual bleeding, the red cell count fluctuated as in normal mature rhesus macaques.

II. *Water retention in relation to œstrogenic stimulation in the rhesus monkey*

(*a*) *Excretion of urine by normal mature female rhesus monkeys.* Cyclical variations in the daily water balance of the female pig-tailed monkey are so pronounced as to reveal themselves in regular fluctuations both in body weight and in the amount of urine excreted daily, even when the animal is not on a controlled diet. Daily observations of body weight and urinary excretion were made on three normal rhesus monkeys (139, 167, 168), each of which was observed through two menstrual

cycles. As stated in our preliminary note [Guthkelch & Zuckerman, 1937], cyclical fluctuations in body weight were not observed, while cyclical variations in the amount of urine excreted, of the kind manifested by the pig-tailed monkey, were detected only in 168 (see Table II). It is of interest that 168 was abnormal in showing relatively extensive swelling of the sexual skin (as judged by the normal standard for the rhesus monkey).

TABLE II. Urine excretion and approximate water balance in a normal mature female rhesus monkey (168)

Cycle number	Days of cycle	Average daily urine excretion c.c.	Estimated daily water balance c.c.
1 (incomplete)	8–14	191	+44
	15–22	267	−55
	23–29	219	− 6
2	1–7	254	−35
	8–14	187	+18
	15–21	278	−77
	22–30	248	−31
3	1–7	190	+ 3
	8–14	171	+26
	15–21	196	−13
	22–31	197	− 8

(*b*) *Excretion of urine by spayed rhesus monkeys injected with œstrin.* Sexual-skin activity occurs during that phase of the menstrual cycle in which œstrogenic hormone is dominant, and can be stimulated experimentally both in ovariectomized and in normal animals (e.g. the pig-tailed monkey during the second half of the cycle) by the injection of œstrin. The retention of water was therefore investigated in three spayed rhesus macaques (180, 181, 183) during eight courses of injections (see Table III).

The animals were given the following constant mixed diet daily, and the food residues and fæces of each animal were collected and weighed every morning:

Apple	110 g.	Orange	60 g.
Banana	45	Potato	165
Bread	50	Rice	175
Carrot	100		

No free water was given during the course of the experiments, which were otherwise conducted in the same way as that upon the pig-tailed monkey reported previously [Krohn & Zuckerman, 1937].

Considerable fluctuation occurred in the volume of urine excreted daily, and in only three of the eight experiments did the average daily

TABLE III. Urine excretion and approximate water balance in spayed rhesus monkeys

Animal	Period of experiment	Amount of œstrone injected daily	Days	Mean daily urine excretion c.c.	Estimated daily water balance c.c.
180	1	Nil	1–8	160	0
	2	100γ	9–31	184	− 7
	3	200γ	32–40	192	− 19
	4	400γ	41–58	232	− 61
	5	1000γ	59–72	232	− 67
	6	Nil	73–79	240	− 76
	7	Nil	80–86	163	− 3
181	1	Nil	1–8	165	− 6
	2	100γ	9–22	210	− 42
	3	100γ + 5 R.U. progestin	23–36	224	− 74
	4	Nil	37–50	238	− 80
	5	400γ	51–64	180	− 46
	6	Nil	65–71	230	− 97
183	1	Nil	1–14	164	− 22
	2	100γ	15–28	131	− 2
	3	Nil	29–42	183	− 30
	4	200γ	43–56	159	− 26
	5	200γ + 10 mg. testosterone propionate	57–70	154	− 13
186	1	Nil	1–14	190	− 20
	2	2 R.U. progestin	15–28	115	+ 28
	3	Nil	29–42	85	+ 32
	4	2 R.U. progestin	43–56	83	+ 48
	5	Nil	57–74	107	+ 17

amount of urine decrease during, and increase after, the period of injections (181, periods 4–5–6; 183, periods 1–2–3–4, Table III). 180, which failed to show any changes in urine excretion in relation to the administration of œstrin, nevertheless showed a greater amount of sexual-skin swelling in response to the treatment than did any of the other animals.

Most urine is excreted by the pig-tailed monkey during that phase of the menstrual cycle when the effect of œstrin is least and the effect of luteal hormone greatest. No change was, however, observed in the mean daily excretion of spayed rhesus monkeys injected with either progesterone alone (186, Table III) or with progesterone and œstrone (181·3, Table III), or with œstrone and testosterone propionate (183·5, Table III).

(*c*) *Variations in the daily water balance of rhesus monkeys.* By means of the constants derived in the previous study on the pig-tailed monkey [Krohn & Zuckerman, 1937] for the evaporation of water from urine and food, approximate figures were obtained as follows for the amount of water retained by each rhesus monkey daily (see Tables II and III).

(*a*) Wt. of mixed diet = 705 g.
(*b*) Mean water content of diet = 0·705 g. water/g. solid.
Mean metabolic water = 0·111 g./g. solid.
Approximate total water available from daily diet = 575 g.
(*c*) 100 g. food refuse (*R*) after evaporation in cage ≡ 130 g. original food.
Total water value of food refuse (including metabolic water)
$= (0{\cdot}705 + 0{\cdot}111) \times \frac{130}{100} R$ approximately, $= 1{\cdot}06\ R$ g.
(*d*) Approximate daily water intake = 575 − 1·06 *R* g.
(*e*) Approximate calorific value of food given = 1 cal./g.
Cal. = 2·42 × insensible water (I.W.) [Peters, 1935].
Approximate mean I.W. per day $= \frac{705 - 1{\cdot}3\ R}{2{\cdot}42} = 291{\cdot}3 - 0{\cdot}537\ R.$
(*f*) Urine excreted = urine collected × 1·43 (correction for evaporation) per diem.
(*g*) Water balance = (*d*) − [(*e*) + (*f*)].

The differentiation of the follicular and luteal phase in the pig-tailed monkey presents no difficulty because of the sharp changes that occur in the state of the animal's sexual skin. The rhesus monkey provides no such aid to the analysis of the cycle, and the follicular and luteal phases have to be arbitrarily demarcated by some point in the middle of the cycle (the time of ovulation, if it occurs). For the purpose of the present analysis of the water balance of normal rhesus monkeys, mean figures were as a rule taken for the daily water balance in each week of the cycle.

The results with the normal animal disclose a cyclical variation in the water balance of only 168 (Table II). A change in the water balance was observed about the mid-point of two of the three cycles of this animal that were studied. The point of transition corresponds to the approximate time of ovulation in this species, and to the onset of sexual-skin subsidence in the pig-tailed monkey. Table II shows that in the three cycles investigated, more water was excreted in the second half than in the first half of the cycle, and that water was retained during part of, or throughout, the earlier half of all three cycles.

With the spayed animal, although the methods followed were inadequate for the exact determination of changes in water balance, the results demonstrate certain trends which are in accord with expectation. Thus the daily loss of water during a period of œstrin injections in 181 (period 5, Table III) was doubled during the week after their cessation (period 6). A similar change is observed in periods 1, 2 and 3 of 183. In another experiment (181, periods 2–4), a fortnight's course of œstrin injections was followed by a course of œstrin and progestin of similar duration. During the third fortnight no injections were given. The daily water loss in both the second and third periods was approximately the

same, and in each case nearly double that which was observed during the first fortnight, when œstrone alone was given. In yet another series (183, 4–5), œstrin administration for a fortnight was followed by œstrin and testosterone propionate for the same period. No increase in the loss of water was observed during the second fortnight.

The relative inadequacy of the methods followed in the determination of changes in the water balance of the rhesus macaque (as compared with their value in similar studies on the related pig-tailed macaque) may be due to the lack in this species of monkey of a sexual skin that swells appreciably. Furthermore, rhesus monkeys, because of their great activity, are difficult to keep under control. They scatter and soil their food, and in general are ill-adapted for such metabolism work as has to continue for prolonged periods.

Discussion

The cyclical changes in those primate species which possess a sexual skin that swells (e.g. the chimpanzee, the baboons, the pig-tailed monkey) suggest that water retention is related to phases of œstrogenic stimulation. This inference was shown to be correct in the case of the pig-tailed monkey [Krohn & Zuckerman, 1937], and the generalization has more recently been found to apply also to mammalian species which do not possess a sexual skin—the dog [Harrop & Thorn, 1937] and the rat [Selye, 1937]. Cyclical changes in water metabolism, as noted above, are much less marked in the rhesus than in the pig-tailed macaque, a difference that is probably mainly due to the absence of appreciable sexual-skin swelling in the rhesus.

In spite of this difference between the two species in the character of the sexual skin, the cyclical changes that occur in the concentration of red cells are of the same order in both. Moreover, observation shows that the concentration of the red cells increases during courses of injection of œstrone, a change which is presumably an index of the dehydration of the blood. An increase in the number of red cells from $4\frac{3}{4}$ to $5\frac{1}{4}$ million in an animal weighing 5 kg. implies the removal of about 40 c.c. of plasma from the blood—assuming that the actual number of cells does not increase. Such an amount of fluid could be deposited in the subcutaneous tissues of the diffuse sexual-skin area of the rhesus monkey without revealing itself by any conspicuous swelling.

The protein concentration of the blood plasma was estimated by Dr R. B. Fisher of the Department of Biochemistry, Oxford, on several occasions when blood counts were made. No apparent correlation was, however, found between protein and red cell

concentration, and it was therefore concluded that adjustments in the former are made as rapidly during the course of the "menstrual" hydration and dehydration of the blood as they occur in conditions in which the plasma protein concentration is altered [e.g. see Smith *et al.* 1920; Melnick *et al.* 1936].

In view of the slight changes which occur in the daily water balance of the rhesus monkey during phases of œstrogenic stimulation, it is possible that such fluid as is sometimes deposited in the sexual skin of this species during its phases of activity is derived in the main from the blood itself, and not, as in the pig-tailed monkey, from external sources. Since the concentration of the blood also varies cyclically in the pig-tailed monkey, it is not unlikely that the cyclical retention of extraneous water represents a secondary mechanism related to the more pronounced swelling shown by this species.

Cyclical changes in the red cell count such as occur in the monkey during the menstrual cycle have been stated by some workers to take place in women, but have been denied by others. There is a pronounced diurnal variation in the blood count of women at all times [Leverton & Roberts, 1936; Smith, 1936]. This type of variation, which may be due to differences in diet, exercise, and daily routine, and which were guarded against in our own study on monkeys, possibly obscures any regular "menstrual fluctuation" of the red cell count in women.

Summary

1. The red cell count in both the normal rhesus and pig-tailed macaques is lowest at the beginning and end, and highest in the middle of the menstrual cycle. The fall in the count coincides with the time of ovulation and the time of sexual-skin subsidence.

2. The count rises during and falls after periods in which œstrin is injected into spayed rhesus monkeys.

3. The retention of water in the normal and experimental rhesus monkey during phases of œstrogenic stimulation was demonstrated in some cases in spite of the absence in this species of a sexual skin that swells.

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THE ACCLIMATIZATION OF MICE TO ATMOSPHERES CONTAINING LOW CONCENTRATIONS OF CARBON MONOXIDE

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THERE is evidence that mice, like many other experimental animals, develop some degree of acclimatization to atmospheres containing carbon monoxide. This has been demonstrated by subjecting mice to continuous exposure [Campbell, 1929, 1933, 1934], and to repeated short exposures [Killick, 1933], to atmospheres containing low concentrations of CO.

There is some disagreement as to the correct definition of acclimatization; Campbell [1934] has claimed that the term ought to be defined as maintenance of growth, body weight, appetite, general well-being and fertility; the term, however, is often applied more freely to the absence of positive symptoms of poisoning, and it will be used in this wider sense in the following account of experiments.

It is now an established fact that most experimental animals, when exposed continuously to an atmosphere containing CO, develop a high red cell count and a high hæmoglobin percentage. This has been demonstrated in guinea-pigs [Nasmith & Graham, 1906], rabbits [Campbell, 1929, 1934] and mice [Campbell, 1934]. But this rise in red cell count does not seem sufficient to account for all the phenomena of acclimatization. It has been suggested [Haldane & Priestley, 1935] that acclimatization involves the power of the lungs to maintain the O_2 tension in the arterial blood by secreting O_2 from the alveolar air into the blood. The following experiments were planned to test this hypothesis in the case of mice, and to attempt the elucidation of other factors concerned in acclimatization.

Three groups of young mice were exposed continuously to an atmosphere containing a low concentration of CO. The mice lived in a

large tank with an airtight lid containing a panel of glass through which the animals could be observed. CO_2 absorbent was spread on a tray beneath the perforated zinc floor of the tank, and O_2 entered through a Müller valve to replace that used up by the animals. The concentration of CO used was never sufficient to cause symptoms; at the beginning of the experiment the mixture in the tank contained only 0·03 p.c. CO, and the concentration of CO was gradually increased as the animals became acclimatized. The period of the experiments varied from 6 to 15 weeks. The mice were weighed at regular intervals, and blood was taken from a tail vein for red cell counts and in some cases for reticulocyte counts.

Results

Although the concentration of CO was never raised to a level sufficient to cause definite symptoms of poisoning in the mice, they were somewhat sluggish in their behaviour, their growth was impeded, and the mortality was high. They cannot therefore be described as completely acclimatized to the atmosphere in which they were living; they had, however, developed a considerable tolerance for CO, as evidenced by comparison with control animals, and also by the change in their own response to a test exposure. Table I shows the figures for mortality in the three

Table I. Mortality during exposure to CO

Group	No. of mice	Duration of exposure weeks	Final concentration of CO p.c.	Deaths	Time of deaths
A	16	6	0·245	7	5 during first 3 weeks
B	20	8	0·285	14	11 during first 2 weeks
C	20	15	0·20	14	2 during first week. 12 between 5th and 15th week

groups of mice; in Groups A and B the mortality during the early weeks was high, probably because the concentration of CO was increased too rapidly.

Symptoms

General comparison with controls. The early symptoms of CO poisoning in mice are indefinite, but when they are exposed for the first time to concentrations above 0·15 p.c. CO many animals show an obvious and dramatic "collapse" (described by Haldane [1895]). Their legs appear to give way suddenly, and they lie prone and apparently helpless. Animals often recover and walk about again, although the atmospheric concentration of CO remains the same, but the occurrence of this "collapse"

forms an easily observed sign of poisoning. Table II shows the proportion of animals that "collapse" during their first exposure to various concentrations of CO in air. In this table figures are shown also for the

TABLE II. Exposure of non-acclimatized mice to CO

p.c. CO	No. of mice	No. collapsed	p.c. collapsed	No. killed at end of exposure	p.c. blood saturation with CO when killed average
0·15	33	3	9	3	61·5
0·16	9	0	0	6	62·5
0·20*	124	81	65	28	66
0·22–0·23*	20	15	75	1	56
0·25*	25	22	88	3	68·5 (1 died at 58 p.c.)
0·30*	13	8	62	13	75
0·2 –0·3	182	126	69·3	—	—

* These four groups appear as one group in the last line.

percentage saturation of the blood with CO; the figures represent the degree of saturation found after time had been allowed for the animals' blood to reach equilibrium with the air breathed. The average values for percentage saturation of the blood refer to only a small number of mice, because the percentage saturation could only be estimated in those animals killed at the end of the exposure to CO. The individual variations in sensitivity to CO are so great that it is almost impossible to lay down any general standard, but where one has a large number, such as the 124 mice exposed to 0·20 p.c. CO, these variations tend to cancel one another; of this total of 124 animals 81 (65 p.c.) collapsed during their first exposure to 0·20 p.c. CO; of the total of 182 animals exposed to concentrations of CO between 0·20 and 0·30 p.c., 126 (69·3 p.c.) collapsed. These figures indicate that the majority of unacclimatized mice are severely affected by atmospheres containing over 0·20 p.c. CO.

These figures for unacclimatized mice may be compared with the behaviour of Groups A, B and C after some weeks of continuous exposure to such concentrations of CO.

Group A (16 mice). After 3–4 weeks 10 mice were living in 0·22–0·25 p.c. CO without symptoms of poisoning.

Group C (20 mice). After 9 weeks 11 mice were living in 0·20 p.c. CO with no symptoms of poisoning.

(Group B is omitted because only a small number survived to be exposed to concentrations above 0·20 p.c. CO.)

In view of the variation in sensitivity to CO amongst unacclimatized mice, it may be asked whether these results are due to acclimatization, or merely to the survival of the more resistant individuals.

In Groups A and B the direct evidence of acclimatization rests on a comparison with unexposed control mice; controls were put into the tank with the experimental mice at intervals during the period of continuous exposure; without exception they were more severely affected by the CO than the experimental mice, at whatever period of the exposure they were put in. All the control mice put into an atmosphere containing 0·15 p.c. CO, or more, died in the course of a few hours or a few days, according to the concentration of CO in the tank when they were put in.

TABLE III

Description of mice	No. of mice	p.c. CO in air when mice were killed	p.c. blood saturation with CO average
Groups A and B	3	0·056	43·5
	5	0·08	53·5
	2	0·10	60
	2	0·115	63
	3	0·14–0·167	66
	3	0·22	68
	5	0·245	71·5
	4	0·285	76
Controls	1	0·06	40
	1	0·15	70
	3	0·18–0·19	72
	1	0·22	67

The percentage of COHb in the blood of these controls at the time of their death was no higher, on the average, than that of the experimental animals killed after living in the same concentration of CO. The difference in their symptoms, however, was marked; the control animals were weak, and collapsed after any attempt to run about, while the experimental animals appeared normal.

In choosing the mice for Group C an attempt was made to exclude animals that showed a very high or a very low sensitivity to CO. A number of mice were exposed to 0·20 p.c. CO for 10 min.; a few appeared moribund at the end of this time, and two were only slightly affected; these mice were discarded, leaving a group whose reaction to CO was fairly uniform. All the mice of this Group (C) showed severe symptoms during the test exposure, so severe that the tank was opened after 10 min., because it was feared that many of the mice would die if the exposure were continued; 16 of them collapsed during the first 8 min., and none of them could walk without dragging its legs at the end of the exposure. Yet after 9 weeks' continuous exposure 11 of these mice were living in an atmosphere containing 0·20 p.c. CO without any apparent symptoms of poisoning.

A comparison may be made between the 6 mice of Group C and 7 control mice, all of whom were killed after an exposure of 1 hr. The blood of these mice was equilibrated after death with mixtures of air and CO, so that the value of K (the CO/O_2 partition constant) is known in each case, in addition to the atmospheric concentration of CO and the percentage of COHb in the blood at the time of death. The values for K are shown in Table IV, they actually average 213 for the exposed mice, and 214 for the controls. Although the percentage of COHb in the blood at any of the three CO concentrations used was not significantly higher in the controls than in the exposed mice, yet the symptoms were very different.

After 1 hr. exposure to 0·30 p.c. CO:

2 control mice were moribund.

2 acclimatized mice were quiet, but not seriously affected.

After 1 hr. exposure to 0·20 p.c. CO:

3 control mice had collapsed and were very weak.

2 acclimatized mice were unaffected.

After 1 hr. exposure to 0·15 p.c. CO:

2 control mice had collapsed and were weak.

2 acclimatized mice were unaffected.

These results demonstrate that acclimatization had occurred, for the reaction of the mice to CO had altered as a result of continued exposure to the gas, although the relationship between the atmospheric concentration of CO and the percentage saturation of the blood had remained the same.

Weight curves

Figs. 1 and 2 show the average body weights of the experimental animals (Groups B and C), expressed as a percentage of their average weights at the beginning of the experiment. It will be seen that in each group the average weight either remained stationary or fell slightly during the experimental period.

The weights of Group C may be compared with a group of control animals from the same stock (Fig. 3); these controls were of approximately the same age as the experimental animals, and received similar food.

Blood cell counts

During the experimental period red cell counts and reticulocyte counts were made at approximately weekly intervals on all the mice. The blood was taken from a tail vein; a red cell count was made in the

usual way; the reticulocyte count was made from a smear vitally stained with brilliant cresyl blue. The results of these blood examinations are shown in Figs. 1 and 2. The figures show only the percentage increase in the cell counts, and it may be of interest to state some of the actual

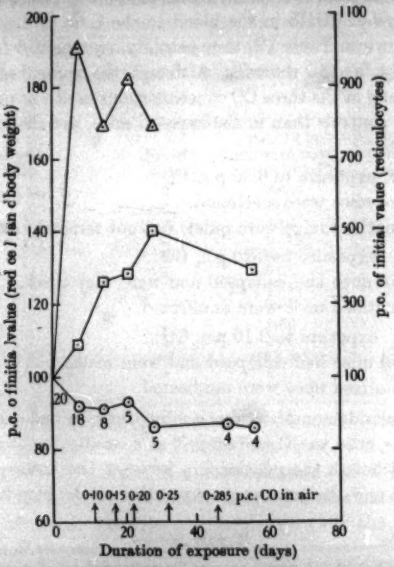

Fig. 1. Group B. ⊙ Average body weight, as percentage of initial weight. ⊡ Average red cell count, as percentage of initial count. ⧋ Average reticulocyte percentage, as percentage of initial value. The figures under the points on the weight curve refer to the number of mice in the group.

figures. The red cell count of the normal mouse varies between 9 and 13 millions per c.mm. with an average of about 11·5 millions; during continuous exposure to small concentrations of CO the average count often rose to 16 or 17 millions, with several individual mice showing red cell counts of 18–20 million cells per c.mm. The reticulocyte count of the normal growing mouse is rather high, compared with the count in human blood; the average is about 4·1 p.c. of reticulocytes among the total red cells; this proportion rose sometimes to 25–30 p.c. of the total red cells during continued exposure to atmospheres containing CO. The

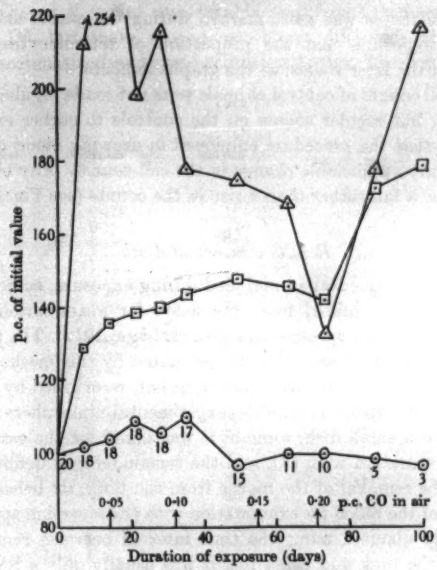

Fig. 2. Group C. Explanation as for Fig. 1.

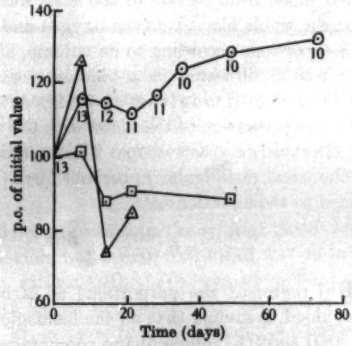

Fig. 3. Controls. Explanation as for Fig. 1.

rise in reticulocytes was most marked during the earlier stages of the continuous exposure, and the proportion of reticulocytes often fell somewhat in the later stages, as the graphs indicate.

Blood cell counts of control animals were not made regularly in these experiments, but regular counts on the controls to earlier experiments have shown that the procedure employed in drawing blood does not of itself cause any appreciable change in the cell counts. Any change that is produced is a fall rather than a rise in the counts (see Fig. 3).

Results obtained at death

When an experimental animal died during exposure, sufficient blood could usually be obtained from the heart for examination with the Hartridge reversion spectroscope [Hartridge, 1912]. The percentage saturation of the blood with CO was estimated by this method.

All other mice, whether acclimatized or not, were killed by beheading immediately after removal from the experimental atmosphere; the blood was caught in a small dish, some of it was taken for the estimation of percentage saturation with CO, and the remainder was defibrinated by whipping. The removal of the mouse from the tank, its beheading, and the dilution of the blood for examination with the reversion spectroscope occupied only about 1 min.; the time interval between removing the mouse from the tank and beheading it was usually only a few seconds, so there was very little chance that an appreciable quantity of CO could be lost from the blood.

The defibrinated blood from certain of the mice was used to plot a partition curve for the whole blood between oxygen and CO. The blood was divided into 3–6 portions according to its volume, and each portion (of about 0·2 c.c.) was equilibrated in a tonometer at 37° C. with a known mixture of CO and air [Douglas *et al.* 1912]. After equilibration for 1 hr. the percentage saturation of the blood with CO was determined by means of the Hartridge spectroscope. The partition curves thus obtained were of the usual rectangular hyperbola form [Douglas *et al.* 1912] and conformed to the equation

$$\frac{[\mathrm{COHb}]}{[\mathrm{O_2Hb}]} = K\frac{[\mathrm{CO}]}{[\mathrm{O_2}]}.$$

[COHb] and [O_2Hb] represent the proportions of carboxy- and oxy-hæmoglobin in the blood, assuming that all the hæmoglobin is in one of these two forms; [CO] and [O_2] represent the percentages of CO and O_2 in the air of the tonometer.

The value of K varied considerably in different mice, as will be seen in Table IV, but there was no consistent difference in this respect between acclimatized and non-acclimatized mice. The non-acclimatized

TABLE IV

Description of mouse	p.c. CO in air when killed	p.c. blood saturation *in vivo*	K (*in vivo*)	Arterial O_2 calc. p.c.
Group C:				
405	0·115	74	—	—
399	0·13	73·5	—	—
401	0·15	73·5	—	—
402	0·15	72	205	11·95
409	0·15	75	224	11·2
407	0·16	77·5	—	—
393	0·20	80	223	11·1
406	0·20	73	172	12·7
394	0·20	78·5	—	—
400	0·20	84	—	—
402	0·20	72	—	—
404	0·20	79	—	—
398	0·21	77·5	—	—
392	0·30	83	278	17·1
397	0·30	71·5	176	21·0
Group A:				
332	0·245	68	144	16·6
336	0·245	67·5	143	16·85
340	0·245	70·5	158	16·2
342	—	—	118	—
Controls (to Group C):				
414	0·15	67	202	14·9
415	0·15	70	216	13·9
411	0·20	75	211	14·1
412	0·20	74·5	162	9·5
413	0·20	77	245	14·6
416	0·30	80	194	14·5
417	0·30	82	169	11·1

controls appearing in this table were of the same stock as Group C, and are therefore strictly comparable only with the acclimatized animals of this group. When the value of K for an individual mouse was known, it was possible to use the equation

$$\frac{[\text{COHb}]}{[\text{O}_2\text{Hb}]} = K\frac{[\text{CO}]}{[\text{O}_2]}$$

to calculate the O_2 pressure in the arterial blood during life; assuming that the hæmoglobin of the arterial blood was all in the form of COHb or O_2Hb, the ratio COHb/O_2Hb was obtained from the percentage saturation with CO of the blood when the mouse was killed; after an exposure of 1 hr. or more to air containing a constant concentration of CO, it was assumed that the pressure of CO in the air breathed, in the

alveolar air, and in the blood, was the same; the value of O_2 in the equation, therefore, is the pressure of O_2 in the arterial blood.

In the majority of animals the arterial O_2, calculated in this way, falls within the range one would expect, assuming the alveolar O_2 of the mouse to be not very different from that of the human being (see Table IV).

Tables III and IV show that there was considerable variation in the relationship between the percentage saturation of the blood at death, and the atmospheric concentration of CO, in both acclimatized and non-acclimatized mice. This merely confirms the observation of great variations in the value of K; there is no evidence that acclimatization alters this relationship. The high mortality amongst the mice exposed continuously to CO is important in this connexion; it is likely that the animals whose blood has a high relative affinity for CO will be eliminated early. Any tendency for the percentage saturation figures for the acclimatized mice to be lower than those for control mice, under comparable conditions of exposure, might be explained by this selective process, killing off the more susceptible animals.

Post-mortem findings

Considerable variations were observed in the size of the spleen; most of the acclimatized mice had spleens that were larger than those of controls. Table V shows the weight of spleen per 10 g. body weight in

TABLE V

History of mice	No. of mice	Spleen weight in mg. per 10 g. body weight		
		Average	Maximum	Minimum
Continuous exposure to CO, 7–30 days	19	151	336	68
Continuous exposure to CO, more than 30 days	26	262	524	66
Killed after 1 hr. or less exposure to CO	74	82·6	197	23
Killed without exposure to CO*	99	97	354	23
Normal mice	5	81·4	165	35

* Many of these mice were used for toxicity tests, and therefore cannot be considered normal.

the experimental mice and in control animals. These figures are evidence of a considerable splenic enlargement in the mice acclimatized to CO. Histological examination of the spleens from the acclimatized animals revealed that the main change was an increase in the amount of lymphoid tissue, in some cases so marked that the regular arrangement of the malpighian corpuscles was destroyed. In many of the experimental animals the heart appeared to be enlarged, but no routine weighings

were done of this organ, so that this impression cannot be confirmed by figures.

In the earlier experiments it was noticed that the acclimatized animals when beheaded bled much more freely than the controls; it was found that sufficient blood was obtained from each acclimatized mouse to enable four or more equilibrations to be carried out, whereas it was often only with difficulty that three points could be ascertained for the partition curve of a control mouse.

In the last experiment (Group C) the animals were beheaded in the usual way, the blood caught in a small basin, defibrinated by whipping, and measured. The volume thus measured does not of course represent the true blood volume, but it provides a rough approximation. The blood obtained in this way from six acclimatized mice of Group C varied from 1·4 c.c. from a mouse weighing 21 g. to 2·3 c.c. from a mouse weighing 31 g.; the average volume of blood per 10 g. body weight was 0·77 c.c. The blood from seven of the controls to this group varied from 0·6 c.c. from a mouse weighing 33·5 g. to 1·05 c.c. from a mouse weighing 43 g.; the average volume of blood per 10 g. body weight was 0·20 c.c. Although it is obvious that the volume of blood measured in this way may be dependent on many factors other than the actual volume of the circulating blood, yet the difference between acclimatized and non-acclimatized animals is striking. In considering the significance of these measurements it should be remembered that acclimatized and non-acclimatized animals were killed under comparable conditions, i.e. with their blood between 65 and 85 p.c. saturated with CO.

Reference has already been made to the increase in the red cell counts of the acclimatized mice; if these rough measurements of the blood collected by beheading the animals do indicate a corresponding increase in blood volume, then the total O_2 capacity of the circulating blood in these mice must have been greatly increased.

Discussion

In general the results described confirm the conclusions of Campbell [1929] that when mice become acclimatized to carbon monoxide the main change is an increase in their red corpuscles and hæmoglobin, and that the relationship between the atmospheric concentration of CO and the percentage saturation of the blood remains unaltered. Acclimatization in these mice seems to consist in a changed relationship between the degree of saturation of the blood with CO and the severity of the symptoms produced.

Campbell [1932] described a hypertrophy of the heart muscle in animals exposed continuously to atmospheres containing CO, and considers that the heart muscle is the "chief factor determining the power to tolerate severe CO poisoning". This hypertrophy of the heart muscle is interesting in connexion with the indications of considerable increase in total blood volume in the acclimatized mice. The hypertrophy may be a reaction not only to increased blood viscosity but also to increased blood volume. It seems probable that the increase in total blood volume, if confirmed by more accurate estimations, is a secondary effect of the increase in red corpuscles. Hypertrophy of the heart muscle was also observed by Van Liere [1936] in guinea-pigs kept for considerable periods at an atmospheric pressure of 380–440 mm. Hg. He found that with such treatment the heart-weight body-weight ratio was increased to 155 p.c. of the control value, and suggested that this increase might be due to a work hypertrophy, or an injury hypertrophy, or both.

The changes in the structure and size of the spleen were fairly constant in all three groups of mice, but it is difficult to see why a chronic anoxæmia should cause an increase in lymphoid tissue. It may be, however, that this reaction is peculiar to mice, for it is often said that mice respond to many varieties of trauma by an overgrowth of lymphoid tissue. Van Liere [1936], however, described a very marked splenic enlargement in guinea-pigs kept at a low atmospheric pressure, and suggested that the cause might be the increased numbers of red corpuscles which the spleen was required to destroy. In this connexion an observation of Muira [1936] is of interest; he found that the circulating blood volume of rabbits decreased as the immediate result of acute carbon monoxide poisoning, the diminution in the erythrocyte volume being considerably greater than that of the plasma. He suggested that this fall was due to the taking up by the spleen of those red corpuscles containing carboxyhæmoglobin.

There is no evidence in these experiments with mice that acclimatization is accompanied by the secretion of oxygen in the lungs. Table IV shows the results obtained from the mice whose blood was examined *in vitro* for the value of the constant K; the arterial oxygen pressure, calculated from these figures by the method introduced by Douglas & Haldane [1912], varies from 9·5 to 21·0 p.c. With one exception, therefore, these results are consistent with the hypothesis that diffusion alone was responsible for the transference of oxygen across the alveolar membrane. The one high result may be due to a slight delay in killing the animal at the end of the final exposure, thus allowing time for the

blood saturation to fall slightly; if this did happen the 71·5 p.c. saturation would be below the actual value, and so would give too high a figure for the arterial oxygen.

It is interesting to compare the values for the constant K in this series of mice with those calculated from the figures given by Douglas & Haldane [1912].

	No. of mice	Value of K		
		Average	Maximum	Minimum
Douglas & Haldane	16	204	306	134
From Table IV	17	191	278	118

The range of variation between individual mice is very great, but the limits in the two series are quite close.

When mice are subjected to continuous exposure to atmospheres containing carbon monoxide they fail to exhibit the phenomenon described in the human subject as the result of intermittent exposure to the gas [Killick, 1936]. In the human subject there was no appreciable increase in the red corpuscles or hæmoglobin as acclimatization developed, but there was a change in the degree of blood saturation reached during repeated exposures to the same atmospheric concentration of carbon monoxide.

It is not as yet possible to decide whether this marked difference in reaction to carbon monoxide indicates a difference between the human being and the mouse, or whether continuous exposure and intermittent exposure differ in their effects on the subject.

Summary

1. Three groups of young mice were subjected to continuous exposure to an atmosphere containing a low concentration of CO, over periods of 6–15 weeks. The concentration of CO at the beginning of the exposure was about 0·03 p.c., and was raised, as the mice became acclimatized, to 0·20 p.c. or more.

2. Clear evidence of acclimatization was obtained; the acclimatized mice behaved normally in a concentration of CO that caused severe symptoms in control animals.

3. Acclimatization was accompanied by a marked increase in the red cell count, and in the reticulocyte count; there was evidence suggesting an increase in blood volume. The spleens of the acclimatized animals were large.

4. The relative affinity of the blood for O_2 and CO was determined *in vitro* after the mouse was killed; the values obtained agreed with the

percentage saturation of the blood with CO *in vivo*. These experiment suggest that the acclimatization of mice to CO is not accompanied by any change in the absorption of CO in the lungs, due to the secretion of oxygen.

I wish to thank Dr C. G. Douglas for his help and criticism during the preparation of this work for publication.

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THE "AFTER-POSITIVITY" OF STIMULATED NERVE AND ITS RELATION TO THE FIRST PHASE OF THE RECOVERY HEAT PRODUCTION

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The heat production of frog's nerve [Hill, 1933] occurs in three phases, the initial phase, a rapid recovery phase, complete in a few seconds, and a slow recovery phase, lasting many minutes. Gasser [Erlanger & Gasser, 1937, p. 159] has suggested that the rapid recovery phase is connected with the positive after-potential which he and others [e.g. Amberson & Downing, 1929, p. 23] have found to follow the usual negative response to a tetanus or a single shock. According to Hill, in the sciatic nerve of *Rana esculenta* (Hungarian) at 20° C. the rapid recovery process runs to half completion in 2 or 3 sec., to completion (within 1 p.c.) in 20 sec.; and the total heat liberated in it is about equal to the initial heat. In view of Gasser's suggestion it was desirable to determine the time relations of the after-potential on similar nerves and under similar conditions to those employed in the heat experiments.

Method

Preparation. Hungarian frogs (winter) were used, which had been kept at a temperature of 14–20° C. for some days. The sciatic nerves were dissected and placed in Ringer's solution (6·75 g. NaCl, 0·2 g. $CaCl_2$, 0·15 g. KCl per litre) to which phosphate had been added (5 mg. P per 100 c.c.: *p*H 7·2). The Ringer's solution was oxygenated continuously for about 4 hours. One nerve was then taken out, the pelvic end injured by crushing, and the nerve placed in air on wooden electrodes impregnated with Ringer's fluid and connected to calomel half-cells in a paraffin chamber. The chamber was closed by means of a glass cover to which was attached a piece of moist filter paper.

Stimulation. The stimulating electrodes were 5–10 mm. and the lead-off electrodes 10–15 mm. apart. Stimulation was by condenser charges and discharges (6 V, 0·25 μF.) produced by a revolving commutator. The frequency was about 100 per sec. The duration of the stimulus

varied from 2 to 20 sec.; the interval between successive stimuli was approximately 5 min. Most experiments lasted for 1½ hours during which the nerve remained in good condition.

Recording. The lead-off electrodes were connected through the usual potentiometer circuit to balance the injury current and then to a Zernicke galvanometer, type Zd, critically damped with a period of 4 sec. The deflexions were observed on a scale at a distance of 2½ metres. Readings were taken at zero time, maximum negative deflexion, the end of the stimulus (if this differed from the maximum), and every 3 sec. from the end of the stimulus until the galvanometer movement became sufficiently slow, when 6 sec. and finally 30 sec. intervals were substituted.

The possibility of the effects observed being due to polarization at the electrodes, or in the nerve itself, was tested by passing a constant current through nerve and galvanometer for about 30 seconds, giving a deflexion of similar magnitude to that obtained in the experiments. The galvanometer, returning to its base-line, in no case showed an "over-shoot" of more than 2 mm.

Temperature. The experiments were made at temperatures varying from 15 to 20° C. This was slightly lower than in the heat production experiments.

Results

Four results of a 10 sec. stimulus are given in Fig. 1. In each case, starting at 16 sec., i.e. 6 sec. after the end of the tetanus (before which the galvanometer deflexion does not represent accurately the value of the current), there is seen to be a rather rapid fall of the after-positivity, and then a long uniform diminution continuing until, in many cases, the positive deflexion became a negative one. The early rapid fall, complete in 20–25 sec. from the end of the stimulus, is approximately exponential when calculated from the line of the subsequent uniform slow movement. It is half complete in 3·7, 3·3, 3·3 and 4·0 sec. in curves Nos. 1–4 respectively. The average temperature of the present experiments was less than that of Hill's experiments on the heat production, so that a mean time to fall to one-half of 3·6 sec. is in approximate agreement with the time taken (2–3 sec.) for half completion by the rapid phase of recovery heat production. The 20–25 sec. for approximate completion also agrees. It would appear, therefore, that Gasser's surmise is correct and that the rapid recovery phase in the heat production is somehow connected with the positive after-potential: with the provision that the first rapid phase only of the positive after-potential is considered.

The very prolonged fall of the positive after-potential following its first rapid fall may possibly be associated with the second and much slower phase of the recovery heat.

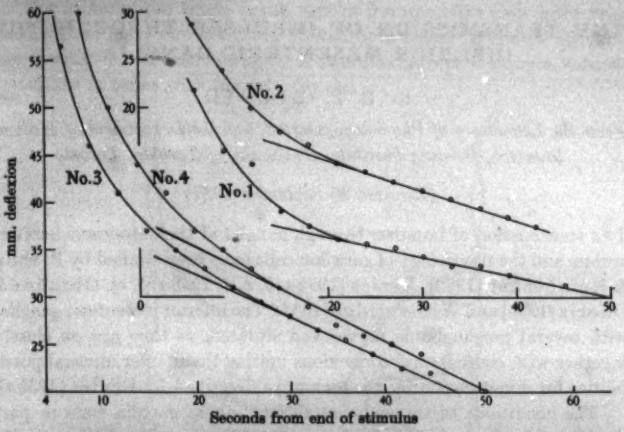

Fig. 1. Positive after-potential following 10 sec. stimulus of nerve, to show two phases, rapid and slow fall respectively. Four nerves at room temperature. The time constant of the rapid fall is discussed in the text. The action potential in every case had been several hundred millimetres in the opposite direction.

Summary

Gasser has suggested that the first rapid recovery phase in the heat production of nerve is somehow connected with the positive after-potential. In nerves similar to those used for the heat measurements there is a rapidly diminishing phase of the after-positivity, which has approximately the same time relations as the first phase of the recovery heat production. The result confirms Gasser's suggestion. It is possible that the prolonged phase of after-potential corresponds to the second slow phase of recovery heat production.

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THE TRANSMISSION OF IMPULSES THROUGH THE INFERIOR MESENTERIC GANGLIA

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(*Received* 20 *September* 1937)

The transmission of impulses through ganglia of the autonomic nervous system and the physiology of ganglion cells have been studied by Bishop & Heinbecker [1932], Eccles [1935*a*, *b*, *c*, *d*, 1936*a*, *b*, *c*], Obrador & Odoriz [1936] and Whitteridge [1937]. The inferior mesenteric ganglia, with several preganglionic nerves and situated, as they are, so closely together with commissural connexions uniting them, offer unusual possibilities for investigation by the technique described by Eccles [1935*a*].

The conditions of transmission through these ganglia were in part elucidated by Langley & Anderson [1895] using the motor activity of the bladder and other pelvic viscera as indicators. A preliminary account of some of the present work has been published [Lloyd, 1937].

Methods

Experiments have been performed on the inferior mesenteric ganglia of thirty-four cats. Of these, twenty were decerebrated under deep ether anæsthesia, the remaining fourteen being anæsthetized with nembutal, 40–45 mg. per kilo intravenously. The observations of Eccles [1935*a*] and Whitteridge [1937] that nembutal has no apparent effect upon the ganglia has been confirmed. The ganglia were exposed for stimulation and recording through a left lateral abdominal incision, without actually entering the peritoneal cavity, thus avoiding undue handling of the viscera. The preparation has been enclosed in a box, the atmosphere of which is kept warm and moist by an evaporator consisting of three 12 V. bulbs covered with strips of gauze, the ends of which dip into a water reservoir.

Action potentials from the ganglion and associated nerves are recorded photographically from a cathode-ray oscillograph driven by a resistance-capacity coupled amplifier with large condensers and grid leaks to

minimize distortion. The image of the oscillograph spot is focused without magnification on the slit of a rising plate camera having a maximum speed of 2 m. per sec.

For stimulation, single break shocks from coreless coils have been used, the primary circuits being broken by a Lucas pendulum synchronized with the camera. Strength of stimulus is controlled by a variable resistance in series with the primary coil.

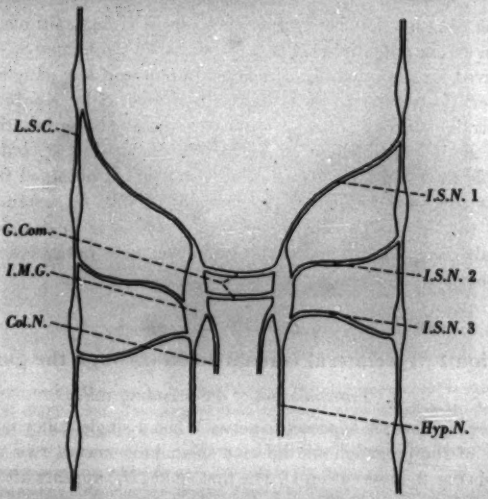

Fig. 1. Semidiagrammatic representation of the connexions of the inferior mesenteric ganglia in the cat. *L.S.C.* lumbar sympathetic chain; *I.S.N.* 1, *I.S.N.* 2, *I.S.N.* 3, first, second and third inferior splanchnic nerves; *I.M.G.* inferior mesenteric ganglion; *G.Com.* interganglionic commissures; *Col.N.* colonic nerve; *Hyp.N.* hypogastric nerve.

Anatomy

The anatomy of the inferior mesenteric ganglia and their associated nerves has been described adequately by Langley & Anderson [1894*a*, *b*, 1895, 1896] and Trumble [1934]. The anatomical observations made during forty-three dissections are in general agreement with these authors, so any detailed description is omitted here. Fig. 1 is a semidiagrammatic representation of the ganglia. The inferior mesenteric ganglion of each side receives usually three preganglionic inferior splanchnic nerves which

leave the lumbar sympathetic chain in the neighbourhood of the 4th, 5th and 6th lumbar ganglia. The inferior mesenteric ganglia of the two sides are connected by the interganglionic commissures lying above and below the inferior mesenteric artery. The postganglionic hypogastric nerves course through the mesentery to reach the pelvic plexuses, while the colonic nerves follow the course of the inferior mesenteric artery.

Nomenclature

Some difficulty has been experienced in ascribing suitable names to ganglion action potentials. It is suggested that the names S_1, S_2, S_3, S_4 be reserved for the synaptically relayed impulses of any ganglion having characteristics similar to those originally described by Eccles [1935*a*]. When straight-through fibres are present it might be satisfactory to use the B_1, B_2, C terminology of Bishop & Heinbecker, but it seems preferable to retain these names to describe waves obtained from nerve alone, as has been done here. Accordingly the D_1, D_2 system has been evolved to describe the direct potentials appearing in ganglion action potentials, i.e., those potentials derived from fibre pathways which are not synaptically relayed in the ganglion.

Results

Section 1. Ipselateral transmission through the ganglion

A. *Transmission of a descending volley*

Recording from the hypogastric nerve. When a single volley is fired into any one of the preganglionic inferior splanchnic nerves two spikes are recorded (Fig. 2, observation 1). The first spike, D_1, appears after a latent period of 1–2 msec. (0·001 sec.) dependent upon the total conduction distance. The second spike, S_2, begins after a latent period of 5–8 msec. The action potential obtained by stimulation of the 2nd inferior splanchnic nerve is invariably larger than that from the 1st, even when the preparation shows a markedly anterior arrangement of its nerves [cf. Langley & Anderson, 1896]. The action potential from stimulation of the 3rd inferior splanchnic nerve apparently varies entirely with the arrangement of the nerves. The D_1 spike varies in amplitude relative to the S_2 spike from one experiment to another, in extreme cases being nearly as great. The lesser degree of temporal dispersion of the D_1 spike contributes to this effect. Often small D_2 spikes are seen interrupting the smooth course of the diphasic artefact. These will be dealt with more fully in Part B of this section.

Recording from the colonic nerve. With the recording leads on the colonic nerve only the S_2 spike appears, beginning after a 5–12 msec. latent period. With colonic nerve leads the action potential has always been found largest on stimulation of the 1st inferior splanchnic nerve. The colonic nerve action potential from a preganglionic volley is shown in Fig. 2, obs. 2.

Fig. 2. Action potentials set up by maximal volleys. The placements of electrodes are given for each observation. The parallel lines represent stimulating electrodes, arrows represent recording electrodes, in each case the grid lead being at the cut, crushed and isolated end of the nerve. Obs. 1 (above) is the ipselateral descending hypogastric nerve potential showing the initial D_1 spike followed by the large S_2 spike. Obs. 2 is the ipselateral descending colonic nerve potential. There is no D_1 spike preceding the S_2 spike. Obs. 3 is the ipselateral antidromic hypogastric nerve potential showing the initial D_1 spike followed by D_2 spikes. The stimulus artefact appears as a break in the resting baseline preceding the action potentials. Time: 1 d.v. = 10 msec.

B. *Transmission of an antidromic volley*

Hypogastric volley, preganglionic recording. Fig. 2, obs. 3 shows the action potential obtained by stimulating the hypogastric nerve while recording from an inferior splanchnic nerve. The record shows the D_1 spike which is always present in both descending and antidromic hypogastric nerve records. In the experiment illustrated, D_2 spikes appear in both descending and antidromic records, though in the former case this potential is largely masked by the coincident and larger S_2 spike. The presence of D_2 spikes in both descending and antidromic records,

and the fact that they are for the most part coincident with the S_2 spike rules out the possibility of their being after-discharge from the S_2 ganglion cells.

Hypogastric volley, colonic nerve recording. In no instance, recording from the colonic nerve, has any action potential been observed from an antidromic hypogastric nerve volley. It may be concluded that there are very few, or possibly no collaterals from direct hypogastric fibres entering the colonic nerve, such as those entering the grey ramus from the 5th lumbar ganglion [Obrador & Odoriz, 1936].

Colonic nerve volley, preganglionic recording. As in the previous instance, no action potential has been recorded. This fact together with the absence of the D_1 spike in the descending record suggests that all, or very nearly all of the fibres in the colonic nerve are truly postganglionic with respect to the inferior mesenteric ganglion.

C. *Analysis by the action of nicotine*

When the ganglion is painted with 0·01 p.c. nicotine, the S_2 spike is removed from the descending hypogastric record, which now appears comparable with the antidromic hypogastric record. The removal of the S_2 spike corresponds to Langley's block and indicates that all the S_2 impulses are synaptically relayed in the ganglion. The appearance of the D spikes in the hypogastric record following nicotine, together with the evidence from antidromic volleys, proves that they are produced entirely by straight-through, or direct fibres. Furthermore, the latent period for these spikes may be accounted for entirely in terms of conduction time (cf. Parts D and E of this section).

Nicotine also entirely removes the colonic nerve action potential, which agrees with other evidence that all the fibres in the colonic nerves are synaptically relayed in the inferior mesenteric ganglion.

D. *Preganglionic conduction*

The action potential obtained by firing a single volley into an inferior splanchnic nerve, and recording from the same nerve, shows two waves, with conduction rates of about 10 and 0·9 m. per sec. for the fastest fibres of each group. These waves correspond to the B_2 and C elevations described by Bishop & Heinbecker [1930] in autonomic nerves, and are named here in accordance with their terminology. The B_2 elevation accounts for a large number of the fibres in the preganglionic nerves and are undoubtedly the fibres responsible for the D_1 spike together with the

preganglionic S_2 fibres. The C elevation is small indicating that there are but few C type fibres. These are the fibres giving rise to the D_2 spikes in the descending and antidromic hypogastric records.

E. *Postganglionic conduction*

The action potential from the hypogastric nerve shows a B_2 and a C elevation as do the preganglionic nerves. The conduction rates vary from 9·66 to 12·8 m. per sec. for the fastest B_2 fibres and from 0·91 to 1·6 m. per sec. for the fastest C fibres. In the hypogastric nerve the relative magnitude of the B_2 and C elevations is reversed from that in the inferior splanchnic nerves. It is clear that the hypogastric nerve contains a much greater percentage of C type fibres.

The B_2 elevation obtained from the hypogastric nerve is in all respects identical with the D_1 spike and the latent period for these two potentials varies only with the total conduction distance, irrespective of whether the ganglion is between the stimulating and recording electrodes, or whether stimulating and recording are carried out on the hypogastric nerve alone. Therefore the fibres giving rise to the B_2 hypogastric elevation must be the straight-through fibres giving rise to the D_1 spike.

The C elevation obtained from the hypogastric nerve alone is characterized by a higher threshold and slower conduction rate. The fibres giving rise to this elevation must be the postganglionic fibres of the inferior mesenteric ganglion together with the C type fibres which are found in the inferior splanchnic nerves and run straight through the ganglion (D_2 fibres).

Adrian *et al.* [1932] described the second or C elevation in the hypogastric nerve and found it to have a conduction rate of 0·8 m. per sec., but were unable to find the more rapidly conducting B_2 component which Erlanger & Gasser [1930] described in the grey ramus of the dog and cat.

F. *Synaptic delay*

Since the D_1 fibres and the S_2 preganglionic fibres are in no way distinct from one another, the synaptic delay of the ganglion may be directly determined by the time difference between the latent periods for the D_1 and S_2 spikes when the earthed lead is on the ganglion itself, the grid lead being on the postganglionic nerve. With postganglionic leads, the time difference is not a measure of synaptic delay, for temporal dispersion is by no means insignificant with fibres of such widely variant conduction rates. The values obtained by this method lie between 4·5 and 6 msec., findings which agree closely with those of Eccles for the S_2 synaptic

delay in the superior cervical ganglion [1935*a*]. This value is the conventional synaptic delay, which is longer than the true value by the time taken for impulses to traverse the fine terminal preganglionic fibres.

Section 2. Contralateral conduction pathways

A. *Transmission of a descending volley*

As in the ipselateral descending hypogastric record, a preganglionic volley in any one of the inferior splanchnic nerves gives rise to a compound action potential in the contralateral hypogastric nerve. This consists of

Fig. 3. Action potentials set up by maximal volleys and recorded from contralateral nerves. Obs. 1 is the contralateral descending hypogastric nerve potential. Obs. 2 is the Sokownin reflex potential. Time: 1 d.v. = 10 msec.

a D_1 spike and an S_2 spike (Fig. 3, obs. 1). The fibres responsible for these two spikes cross from one inferior mesenteric ganglion to the other through the interganglionic commissures. The contralateral D_1 and S_2 spikes are in all respects comparable with their ipselateral counterparts.

B. *Contralateral transmission of an antidromic volley*

Fig. 3, obs. 2 shows the action potential recorded from one hypogastric nerve when a single volley is fired into the contralateral hypogastric nerve. There is no D spike, indicating the absence of any direct fibres. The pathway is thus completely synapsed, the preganglionics being contralateral collaterals from the ipselateral D_1 fibres. These connexions form the pathway for the Sokownin bladder reflex [Langley & Anderson, 1894*a*].

Section 3. Summary of transmission pathways

Fig. 4 illustrates the types of fibres and their connexions which have been demonstrated in the inferior mesenteric ganglia. For simplicity only those fibres associated with one inferior splanchnic nerve have been

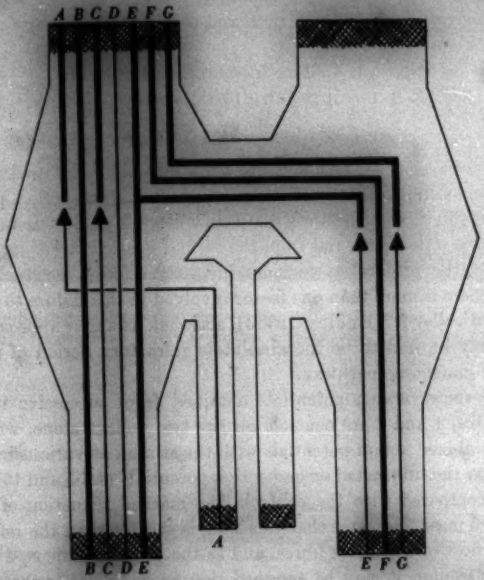

Fig. 4. Diagrammatic representation of the fibres in the inferior mesenteric ganglia. Heavy lines represent myelinated B_2 type fibres, fine lines represent non-myelinated C type fibres. For simplicity only the fibres arising in one preganglionic inferior splanchnic nerve are shown, but all the inferior splanchnic nerves contain similar fibres. A enters the ipselateral colonic nerve, B, C, D and E enter the ipselateral hypogastric nerve, while E, F and G enter the contralateral hypogastric nerve. Full explanation in text.

represented, but there are identical ipselateral and contralateral connexions from the other inferior splanchnic nerves. A accounts for the colonic nerve S_2 spike, its complete removal by nicotine, and the absence of any antidromic record in the colonic nerve. B and E account for the descending and antidromic ipselateral D_1 spike. C accounts for the

ipselateral descending hypogastric S_2 spike, its removal by nicotine and its absence in the antidromic hypogastric record. *D* accounts for the descending and antidromic D_2 spikes. *E* accounts for the Sokownin reflex potential. *F* accounts for the contralateral D_1 spike. *A*, *B*, *C*, *E*, *F* and *G* give the inferior splanchnic nerve B_2 elevation. *D* gives the inferior splanchnic nerve *C* elevation. *B*, *E* and *F* give the hypogastric nerve B_2 elevation. Finally *C*, *D*, *E* and *G* give the hypogastric nerve *C* elevation.

Section 4. The interaction between two preganglionic volleys

A. *Two successive maximal ipselateral volleys*

When two successive volleys are fired into the same preganglionic nerve, the second volley is effective in producing an action potential only when the interval between the two volleys is greater than 3 msec. Further, the action potential from the second volley reaches its full size only when the interval is considerably longer (*circa* 20 msec.). Presumably there is more than one factor involved in this failure to respond to a second volley [cf. Eccles, 1935*a*], although as a first approximation 3 msec. may be said to be the absolutely refractory period of the preganglionic-ganglionic pathway.

Fig. 5 shows action potentials obtained from successive maximal volleys. Obs. 1 and 2 are controls for the two volleys alone, while obs. 3–5 are combined action potentials with the stimuli at various intervals. Fig. 6 shows the subtracted second action potentials at 7·5 and 15·8 msec. intervals compared with the control. The marked reduction of the D_1 spike at 7·5 msec. indicates that this time is shorter than the refractory period of most of the direct fibres, and as the preganglionic S_2 fibres are essentially similar, it may be assumed that preganglionic refractoriness is a factor in the reduction of the S_2 spike at this interval. At 15·5 msec., on the other hand, this factor is probably no longer active, as the D_1 spike is full sized at even shorter intervals. Presumably then the refractory period of the ganglion cells is longer than that of the preganglionic pathway.

In Fig. 6 the earlier onset of the S_2 spike in the subtracted second action potential as compared with the control shows that temporal facilitation [Eccles, 1935*b*] is exhibited by the ganglion cells of the inferior mesenteric ganglion.

Fig. 5. Postganglionic hypogastric nerve action potentials evoked by two maximal successive preganglionic volleys at various intervals, obs. 1 and 2 being controls of the first and second volleys alone.

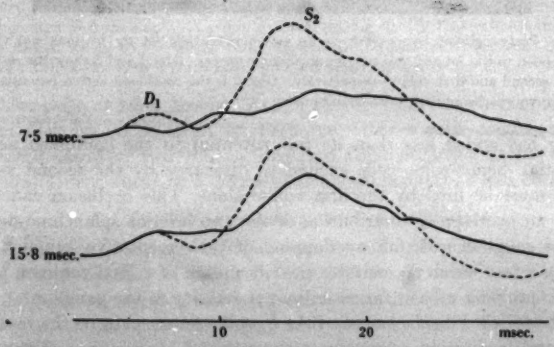

Fig. 6. Enlarged drawing of action potentials (solid lines) evoked by a second volley at 7·5 and 15·8 msec. after a previous similar volley, as determined by subtracting the first action potential from the combined action potentials (cf. Fig. 5). The broken lines show the action potential set up by the second volley alone, all stimuli being synchronized at zero of the time scale.

B. *Two volleys in different preganglionics*

In the inferior mesenteric ganglion the effect of the refractory period of the preganglionic pathway may be avoided by stimulating two inferior splanchnic nerves in succession. Fig. 7, obs. 1 shows the action potential from stimulation of the 2nd inferior splanchnic nerve. Obs. 2 is the combined action potential when the two preganglionic volleys are fired in simultaneously. Obs. 3 is the action potential following stimulation of the 1st inferior splanchnic nerve alone. Fig. 8 shows the course of the subtracted second action potential compared with the control. The second

Fig. 7. Postganglionic hypogastric nerve action potentials set up by maximal volleys applied to the 1st and 2nd inferior splanchnic nerves. Obs. 1 and 3 show the response to second and first volleys respectively. Obs. 2 is the combined action potential, the two volleys being fired in at simultaneity.

volley has added less than its full potential to the combined action potential, hence some cells excited to discharge by the second volley alone must be fired by the first volley alone. This occlusion can only mean an overlapping distribution of the two inferior splanchnic nerves to the ganglion cells (an overlapping of their respective ganglion cell pools), a fact which necessitates the conception of a final common path, and so provides a basis for co-ordinated activity in the ganglion.

The forward displacement of the S_2 spike must again be the result of temporal facilitation occurring at the point of convergence of the preganglionic fibres, i.e. at the ganglion cell.

The extent of occlusion between the ganglion cell pools of two inferior splanchnic nerves is variable, and in one experiment the subtracted and control curves lay within the error limit of analysis.

A number of experiments have been performed in an attempt to demonstrate spatial facilitation (recruitment from the subliminal fringe) by successive volleys in the same, and in different inferior splanchnic nerves. A few experiments have been suggestive, but there is no reliable evidence for spatial facilitation of S_2 ganglion cells in the inferior mesenteric ganglia. It would appear that each ganglion cell receives enough endings from a single preganglionic fibre to cause it to discharge an impulse without the aid of spatial facilitation. On the other hand, some

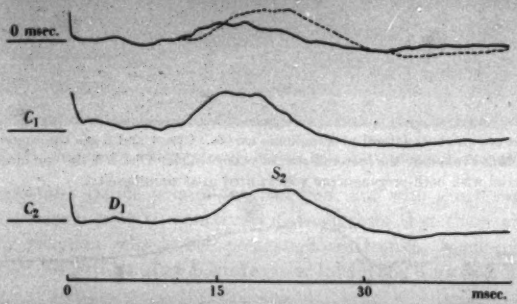

Fig. 8. Curve 1 (solid line) shows the action potential set up by the second volley when the two volleys are fired in simultaneously, as determined by subtracting the first action potential from the combined action potential (cf. Fig. 7). The broken line is the action potential set up by the second volley alone, superimposed for comparison. Curves 2 and 3 show the control potentials from the first and second volleys alone.

of these cells, at least, receive enough endings from some other preganglionic fibre to cause them to discharge. Under such conditions the expected result of two volleys fired into different preganglionic nerves simultaneously would be a degree of temporal facilitation and occlusion.

C. *Two volleys in contralateral preganglionics*

Fig. 9, obs. 1 shows the contralateral descending hypogastric potential. Obs. 2 is the ipselateral potential. Obs. 3 is the potential recorded from the hypogastric nerve when both volleys are fired in at simultaneity. In Fig. 10 similar records have been analysed as in Fig. 8. The subtracted second action potential coincides almost exactly with the control second action potential. The absence of temporal facilitation or occlusion in such experiments indicates clearly that there is little or no overlap of preganglionic fibres from both sides of the body upon ganglion cells of either inferior mesenteric ganglion.

Fig. 9. Potentials set up by maximal volleys as in Fig. 7 except that the stimulating electrodes are on contralateral preganglionic nerves. Obs. 1 and 2 are the control action potentials evoked by the first and second volleys alone. Obs. 3 is the combined action potential with both preganglionic volleys fired in at simultaneity.

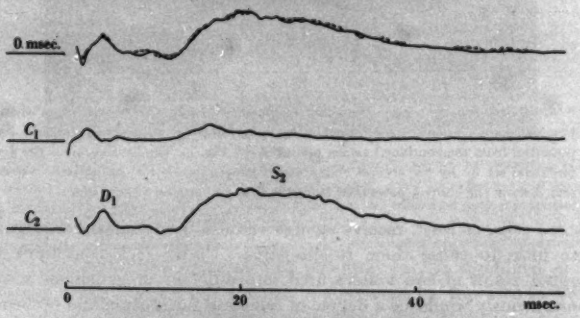

Fig. 10. As in Fig. 8, but for contralateral volleys.

Section 5. Slow potential waves in the inferior mesenteric ganglion

With the earthed amplifier lead on the ganglion itself slow potential waves are recorded as in the superior cervical ganglion [Eccles, 1935*a*, *c*], the 5th lumbar ganglion [Obrador & Odoriz, 1936] and the ciliary ganglion [Whitteridge, 1937]. Fig. 11 shows the ganglionic action potential obtained with the earthed lead on the ganglion and the grid on the hypogastric nerve. After the spike potentials and diphasic artefact

there are the usual *N* and *P* waves. With postganglionic leads, these waves are absent, in agreement with Eccles's findings in the superior cervical ganglion, that the *N* and *P* waves are confined to the ganglion.

Eccles [1935*c*] has shown that there is a decremental spread of slow positivity along a few millimetres of the postganglionic trunk of the superior cervical ganglion. This is not due to outlying ganglion cells, but resembles an electrotonic spread. In the case of the inferior mesenteric ganglion, however, slow waves often appear when the earthed lead is

Fig. 11. Ganglionic action potential evoked by a single preganglionic volley.

well below the visible pole of the ganglion, but with great variability from one experiment to another, which suggests that there are often outlying ganglion cells in the postganglionic trunks. Such cells have been observed by Langley & Anderson [1894*b*] and Kuntz & Moseley [1936].

The N wave

The presence of the *N* wave in the inferior mesenteric ganglion confirms the opinion [Eccles, 1935*c*] that not all the *N* wave of the superior cervical ganglion is associated with the S_1 type of ganglion cell. Furthermore, in the inferior mesenteric ganglion an *N* wave has never been detected in the absence of a spike potential

The P wave

The large *P* wave of the inferior mesenteric ganglion serves to link the *P* process more firmly with the S_2 type of ganglion cell. Further, throughout a range of stimulus strengths the conducted spike and the *P* wave vary together and their thresholds appear to be similar. The parallelism between the spike potential and *P* wave is more satisfactorily shown in a ganglion where there is a uniformity of ganglion cells. It is felt that this parallelism strongly supports the conclusion that there is a common causal factor [cf. Eccles, 1936*a*].

The fact that an *N* wave has never been observed in the inferior mesenteric ganglion in the absence of a spike potential is in line with the fact that there is little or no subliminal fringe in the ganglion. Hence

any preganglionic volley large enough to produce an N wave will produce a spike potential, otherwise there would be some degree of spatial facilitation. The size of the N wave relative to the spike potential and P wave must be determined largely by the extent of the subliminal fringe of any given ganglion cell pool, inasmuch as this zone contributes to the N wave while the spike and P wave depend upon the discharge of impulses by the ganglion cells [Eccles, 1936*a*, *c*]. Furthermore, where there is a subliminal fringe in the ganglion cell pool the N wave will vary not with the size of the spike and P wave alone, when the stimulus strength is varied, but also with the extent of the subliminal fringe. In the inferior mesenteric ganglion the N wave varies with the spike potential and P wave between threshold and maximality (as indicated by the absence of any shift in the point of maximum positivity [cf. Eccles, 1935*c*] and is almost never greater than the P wave in amplitude (with reference to the true base-line) and is usually somewhat less than the P wave. Hence it may be concluded independently from the observed absence of spatial facilitation, that there is little or no subliminal fringe in the ganglion cell pool, and that the small N wave is produced only by those cells which discharge impulses.

Discussion

The ganglion cells of the inferior mesenteric ganglia are clearly of very uniform size and may be classed in one group. This group may be identified with the S_2 group of cells in the superior cervical ganglion [Eccles, 1935*a*] on the following points of similarity:

(1) Latent period.
(2) Preganglionic conduction rates.
(3) Synaptic delay.
(4) Threshold.
(5) Postganglionic conduction rates.
(6) Relative size of N and P waves.

The uniformity of cell size is indicated by the simple form of the S_2 spike in the inferior mesenteric ganglia in contrast to the four distinct S potentials observed by Eccles in the superior cervical ganglion, the great irregularity of the spikes obtained in the 5th lumbar ganglion by Obrador & Odoriz [1936] and the T and T_2 spikes of the ciliary ganglion [Whitteridge, 1937]. Histologically the cells of the prevertebral ganglia are notable for their uniformity of size [de Castro, 1932]. Furthermore, these cells correspond to the middle group of cells in the superior cervical

ganglia [de Castro, 1932] which presumably are responsible for the S_2 spike potential of that ganglion [Eccles, 1935*a*].

The interaction of preganglionic impulses. There is little or no subliminal fringe in the ganglion cell pool of any inferior splanchnic nerve (Sections 4 and 5). It may be concluded that the detonator response [Eccles, 1936*a*, *c*] produced in any cell by a single volley is always sufficiently large to set up an impulse in that cell. It does not follow that a ganglion cell will make synaptic connexion with only one preganglionic fibre (as suggested for T_1 ganglion cells in the ciliary ganglion [Whitteridge, 1937]). Indeed occlusion between the ganglion cell pools of two inferior splanchnic nerves shows that this is not the case. Fig. 12

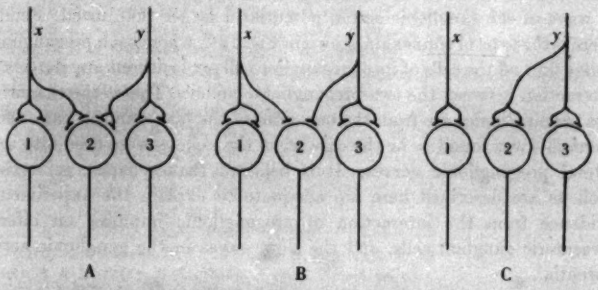

Fig. 12. Diagram to show suggested modes of synaptic relationship between preganglionic endings and ganglion cells. Full explanation in text.

is a simplified schematic representation of the types of synaptic connexion between preganglionic fibres and their ganglion cells, advanced to explain the observed facts of transmission in the inferior mesenteric ganglia. In each case let x and y be two preganglionic fibres impinging upon ganglion cells 1, 2 and 3, and suppose that two endings must be active to set up sufficient detonator response to discharge a ganglion cell, while one active ending would suffice to excite a ganglion cell subliminally (setting up an N wave and c.e.s., but not a conducted impulse or P wave [cf. Eccles, 1936*a*, *c*]. Then in Fig. 12A an impulse in x would fire cells 1 and 2, setting up an N wave, a propagated impulse, and a P wave. An impulse in y would fire cells 2 and 3 in a similar manner. x and y stimulated together would fire cells 1, 2 and 3 with occlusion equal in extent to the amount of potential contributed by cell 2 when either preganglionic is stimulated alone. In no case would there be any

subliminal fringe, and consequent spatial facilitation, since in no case is a single ending on a cell alone active. The excess of endings on cell 2 would presumably provide the required conditions for temporal facilitation. Furthermore, with this scheme the spike potential, *N* and *P* waves would be expected to vary together (Section 5). In Fig. 12B stimulation of *x* would fire cell 1 and set up an *N* wave and subliminal e.e.s. in cell 2. Stimulation of *y* would fire cell 3 and again set up an *N* wave and subliminal e.e.s. in cell 2. Stimulation of *x* and *y* together would fire cells 1, 2 and 3, recruiting 2 from the subliminal fringe by spatial facilitation. The possibility of such events occurring in the inferior mesenteric ganglia must not be ignored, inasmuch as some experiments have indicated a very small subliminal fringe in the ganglion cell pool, and in some cases the *N* wave of the ganglionic action potential is larger than usual. Finally, there is the type of connexion shown in Fig. 12C, where each preganglionic volley fires off the cells of its own ganglion cell pool without any detectable interaction between the two preganglionic volleys. This is the case when the preganglionics are from the two sides of the body, and in one experiment this was found to be the case when the volleys were fired into ipselateral preganglionic nerves. It is believed that synaptic connexions such as are described here are adequate to explain the experimental evidence from the interaction of preganglionic impulses on inferior mesenteric ganglion cells, and the slow waves in the ganglionic action potential.

Summary

The fibre pathways through the inferior mesenteric ganglia have been described on the basis of evidence derived from the action potentials set up by volleys in various preganglionic and postganglionic nerves, together with the action of nicotine in blocking synaptic transmission.

Temporal facilitation is observed after a maximal volley fired into the same preganglionic or between volleys in different preganglionic nerves. Spatial facilitation is not a feature of ganglion cell activity in the inferior mesenteric ganglion. Thus there is no subliminal fringe in the ganglion cell pool.

Occlusion may be demonstrated between the ganglion cell pools of ipselateral preganglionic nerves. There is no occlusion between the ganglion cell pool of an ipselateral preganglionic nerve and that of a contralateral preganglionic nerve.

Slow potential waves are set up in the ganglion cells by preganglionic volleys. The *N* wave varies only as the total number of ganglion cells

actually discharging impulses, further indicating that there is no subliminal fringe in the ganglion cell pool. The *P* wave resembles that associated with the S_2 ganglion cells of the superior cervical ganglion.

A simple scheme is presented to explain the suggested forms of synaptic relations between preganglionic fibres and ganglion cells.

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CHANGES IN CREATINE, PHOSPHAGEN AND ADENYLPYROPHOSPHATE IN RELATION TO THE GASEOUS METABOLISM OF THE HEART

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Recent work has drawn attention to the importance of adenylpyrophosphate in muscular contraction. Lohmann [1934*a*] suggested that the first event in contraction was a breakdown of adenylpyrophosphate and that this was followed by a loss of phosphagen which supplied phosphate for resynthesis of adenylpyrophosphate. He also corroborated Eggleton & Eggleton's [1929] statement that in anaerobic contraction in the skeletal muscle of the frog, 75 p.c. of the phosphagen was lost before any breakdown of adenylpyrophosphate occurred, but that once this level was reached there was a very definite loss of adenylpyrophosphate. In 1934 Weicker showed that the perfused mammalian heart muscle when asphyxiated or poisoned by iodoacetic acid lost almost completely both phosphagen and adenylpyrophosphate. The present investigation was undertaken to determine the comparative loss of creatine, phosphagen and adenylpyrophosphate in cardiac muscle when fatigued, asphyxiated and poisoned with sodium iodoacetate, and also to determine whether in aglycæmia due to work with insulin, similar comparative changes were produced. This meant that the complete gaseous metabolism of heart muscle had to be related to variations in phosphorus fractions, amino nitrogen, carbohydrate utilization, urea and ammonia production.

Methods

Blood sugar was determined by the Hagedorn-Jensen method, corrections being made for the non-carbohydrate reducing bodies, urea by aeration [van Slyke & Cullen, 1916], ammonia by modifications of the Parnas & Heller [1924] and Parnas & Klisiecki [1926] methods.

Glycogen was estimated by the modification described by Good *et al.* [1933], phosphorus fractions, expressed as mg. P, by the method of Eggleton & Eggleton [1929], and the gaseous metabolism of the heart by the method of Cruickshank & Startup [1933*a*]. Amino-acid nitrogen of the blood was determined by the van Slyke manometric apparatus, while that of the heart muscle was estimated by a modification of the method described by Luck [1927]. Creatine was estimated by the colorimetric method described by Peters & van Slyke [1931]. Upon completion of the experiment the heart was immediately removed, weighed and ground in liquid air.

Results

(*a*) *Cat.* I. *The perfused excised heart*

Normal phosphate fractions of the perfused cat's heart are as shown in Table I. Seven experiments were made, the hearts being perfused with oxygenated blood for 1 hr. The results show a close agreement; the

Table I. Excised cat's heart, perfused with oxygenated blood for one hour. All amounts are in mg./100 g. of tissue

	Phosphagen mg./100 g.	Phosphagen index	Orthophosphate	Orthophosphate p.c. increase	Pyrophosphate	Pyrophosphate p.c. decrease	Total phosphorus
Ventricle	4·5	0·132	29·6	18·4	18·4	26·4	79·2
Ventricle	4·9	0·136	31·1	23·6	19·6	21·6	83·7
Ventricle	5·3	0·177	24·8	—	19·9	20·4	83·2
Ventricle	4·8	0·146	28·1	12·4	21·7	13·2	81·3
Ventricle	4·7	0·157	25·2	—	22·8	8·8	78·6
Ventricle	4·6	0·126	32·1	28·0	21·3	14·8	87·7
Averages	4·78	0·146	28·48	12·07	20·45	17·5	83·77
Normal hearts not perfused (two highest values)							
Ventricle	6·2	0·183	26·5	6·0	21·8	12·8	80·8
Ventricle	6·0	0·178	26·7	6·8	22·3	10·8	79·8

phosphagen index, i.e. phosphagen/phosphagen + orthophosphate, varies from 0·126 to 0·177 with an average of 0·146. The phosphagen index of the perfused heart is never so high as that of the non-perfused heart. That this is so is seen from the results obtained on non-perfused hearts in which the phosphagen percentage is of the order of 6·00 mg./100 g. and the index 0·180 (Table I). It is well to remember that experimental figures in this connexion are not necessarily normal figures. It may therefore be taken that the normal heart has 6·00 mg. p.c. of phosphagen and that the optimum index is not less than 0·180. In a similar manner the question arises as to what are the normal amounts of ortho- and pyrophosphate in the mammalian heart. From numerous estimations

of these fractions it may be assumed, and further reference will be made to this point later, that ortho- and pyrophosphate balance each other in amounts of 25 p.c. each. Pyrophosphate seems, in these experiments, to be much more affected by manipulation of the heart than is the ortho-fraction, as is seen from the results in Table I where the percentage increases of the ortho- are contrasted with the percentage decrease of the pyrophosphate. The pyrophosphate as estimated represents two-thirds of the adenylpyrophosphate in the muscle. The effects of progressive asphyxia are shown in Table II; the hearts were perfused with blood in a closed system to which no oxygen was admitted, the perfusions lasting from 1½ to 2½ hr. The results have been arranged according to the phosphagen indexes. We regard the index as evidence of a failing heart whose energy reserves are being diminished, the index giving a clue to the extent of this diminution. While the phosphagen figures do not necessarily determine the length of time of the perfusion they are indicative of the degree of asphyxia. The volume of blood was kept at 200 c.c. The first point of note in Table II is that the phosphagen and

TABLE II. Excised cat's heart, perfused with blood, to asphyxial failure. All estimations on the ventricle in mg./100 g.

Phosphagen mg./100 g.	Phosphagen p.c. decrease	Phosphagen index	Orthophosphate	Orthophosphate p.c. increase	Pyrophosphate	Pyrophosphate p.c. decrease	Total phosphorus
1·3	78·3	0·023	44·2	76·8	9·6	61·6	84·6
1·6	74·0	0·028	42·4	69·6	11·2	55·2	83·7
1·9	68·3	0·045	40·2	60·8	12·2	51·2	84·2
2·0	66·6	0·050	37·5	50·0	9·9	60·4	75·9
2·5	58·3	0·065	35·7	42·8	10·1	59·6	77·2
2·5	58·3	0·066	35·5	42·0	11·8	52·8	80·8
2·6	56·6	0·069	35·0	40·0	13·4	44·4	80·2
2·8	53·3	0·077	33·3	33·2	16·3	34·8	85·7
3·7	38·3	0·089	31·7	26·8	18·5	26·0	81·8

orthophosphate figures bear an inverse relation to each other; the second is that phosphagen breakdown is always greater than the breakdown of pyrophosphate and presumably precedes it, but not to the extent suggested by Eggleton & Eggleton for skeletal muscle; the events are much more closely related the one to the other than they are for skeletal muscle. The most pronounced degree of asphyxia gave a phosphagen loss of 78·3 p.c. and a pyrophosphate loss of 61·6 p.c. with a phosphagen index of 0·023.

(*b*) *Dog.*

Experiments have been carried out on the excised heart and the isolated heart-lung preparation. These experiments must be discussed separately.

The excised heart. The heart of the dog does not lend itself to this type of experiment, the dog's heart suffers very quickly from any lack of oxygen. Small hearts were used and every means taken to maintain an efficient coronary circulation. A well-oxygenated heart beats vigorously, but after an hour such a heart suffers from some degree of oxygen lack. Compared with the normal heart which has not been perfused the excised heart always loses phosphagen under the best experimental conditions possible. It is again necessary to secure standard figures for phosphate fractions from hearts which have been removed from the body and placed at once in liquid air. Phosphate fractions determined on the ventricle of four hearts gave the following average figures: phosphagen 6·20 mg., phosphagen index 0·168, orthophosphate 28·6 mg., pyrophosphate 28·05 mg. per 100 g., and total phosphorus 91·70 mg. (Table IV).

Since under the conditions of perfusion no adequate control for the ventricle is available it was suggested that the auricle, because of its thinner structure, would suffer less from the effects of asphyxia than the ventricle, accordingly phosphate fractions were determined on both structures (Table III). With rapid asphyxia the comparative picture

TABLE III. Excised dog's heart, perfused in a closed system to asphyxial arrest

	Phosphagen mg./100 g.	Phosphagen p.c. decrease	Phosphagen index	Orthophosphate	Orthophosphate p.c. increase	Pyrophosphate	Pyrophosphate p.c. decrease	Total phosphorus
Auricle	5·2	13·3	0·161	27·6	10·4	21·0	16·0	81·1
Ventricle	2·8	53·3	0·070	36·7	46·8	13·1	47·6	85·7
Auricle	4·8	20·0	0·158	27·0	8·0	19·2	23·2	84·2
Ventricle	1·9	68·3	0·046	39·2	56·8	11·0	56·0	89·0
Auricle	4·2	30·0	0·146	27·9	11·6	18·5	26·0	75·0
Ventricle	1·6	73·3	0·038	39·7	58·8	10·6	57·6	79·0

obtained was of interest, for it showed that the failure of the heart was essentially due to changes in the ventricle. That this is so is evident, for the auricle continues to beat vigorously after cessation of all movement in the ventricle. The three experiments quoted in Table III show the progressive nature of the asphyxia. The auricles have an average phosphagen index of 0·15 when that of the ventricles has fallen to 0·05. In the auricles there is little change from the usual figures for 1 hr. aerobic perfusions compared with the ventricles, where a rapid initial fall in phosphagen is closely followed by a rapid fall in the pyrophosphate fraction. Taking the third experiment one notes that a 73 p.c. diminution in the phosphagen is associated with a 58·8 p.c. increase in orthophosphate and a 57·6 p.c. decrease in the pyrophosphate. Such results demonstrate the close interrelation between these phosphorus fractions in the chemical

changes which constitute contraction in the mammalian heart. A repetition of these experiments with the addition of iodoacetic acid to the perfusing blood gave practically the same results.

The isolated heart-lung preparation. Reference has already been made to what is probably the normal phosphagen content of the dog's heart. Exp. 1 in Table IV gives a good average result for phosphorus fractions

TABLE IV. Isolated heart-lung experiments

Exp.		Phosphagen mg./100 g.	Phosphagen p.c. decrease	Phosphagen index	Orthophosphate	Orthophosphate p.c. increase	Pyrophosphate	Pyrophosphate p.c. decrease	Total phosphorus
					Normal				
1	Auricle	7·0	—	0·215	25·5	—	27·6	—	102·2
	Ventricle	6·5	—	0·172	31·3	—	30·1	—	115·3
	Average 4 ventricles	6·2	—	0·168	28·6	—	28·05	—	91·7
					Perfused 1 hr.				
2	Auricle	5·9	—	0·176	28·0	12·0	19·8	20·8	87·5
	Ventricle	5·3	11·6	0·152	29·6	18·4	18·7	25·2	91·7
					Perfused 1–2 hr.—insulin and adrenaline				
3	Auricle	4·8	20·0	0·124	31·1	24·4	15·2	39·2	110·1
	Ventricle	4·0	33·3	0·076	48·3	93·2	13·8	44·8	118·4
4	Auricle	5·2	13·3	0·190	26·8	7·2	19·3	22·8	83·2
	Ventricle	5·0	16·6	0·098	46·0	84·0	10·9	56·4	87·1
5	Auricle	4·0	33·3	0·087	41·6	66·4	5·2	79·2	110·1
	Ventricle	3·1	46·6	0·060	48·3	90·8	3·8	84·8	118·4
					Perfused 1–2 hr. with iodoacetic acid (5 c.c. = 0·025 g.) and adrenaline				
6	Auricle	7·8	—	0·233	25·3	1·2	16·9	32·4	87·6
	Ventricle	4·6	23·3	0·130	30·0	20·0	6·2	75·2	92·7
7	Auricle	4·6	23·3	0·150	27·6	10·4	11·0	56·0	99·4
	Ventricle	4·2	30·0	0·106	35·7	42·8	8·4	66·4	112·7
8	Auricle	4·1	31·6	0·134	26·4	5·6	10·9	56·4	100·8
	Ventricle	3·3	45·0	0·082	36·7	46·8	9·5	62·0	104·9
					Chloroform poisoning				
9	Auricle	2·3	61·6	0·061	35·3	41·2	13·8	44·8	81·1
	Ventricle	2·2	66·6	0·056	36·9	47·6	13·3	46·8	91·4

in the heart of the dog. Beneath it are given average figures for four ventricular estimations which bear out what has already been said concerning normal figures for the phosphorus fractions of the non-perfused heart. The second experiment shows that perfusion for 1 hr. results in a slight loss of phosphagen and pyrophosphate, the pyrophosphate loss being greater than that of the phosphagen. In Exp. 3 the heart was perfused with blood from a dog which was given 5 units of insulin per kilogram of body weight. This was done in order that the heart should rapidly exhaust its store of carbohydrate. To hasten the depletion of blood sugar and cardiac glycogen insulin and adrenaline were

added to the blood at the beginning of every half-hour period. Further data for this experiment are given in Table V. The heart, driven at a rate between 140 and 180 beats per minute with a blood pressure of 120 mm. Hg, utilized blood sugar so rapidly that it fell from 41 to 18 mg./100 c.c. in the hour. From the data in Table IV it will be seen that the fall in phosphagen is 20·0 p.c. for the auricle and 33·3 p.c. for the ventricle. There is an increase in the orthophosphate which amounts to 24·4 and 93·2 for auricles and ventricles respectively. In contrast to this is the decrease in the pyrophosphate, which measures 39·2 and 44·8 mg. p.c. for the auricles and ventricles respectively. The phosphagen indexes for auricle and ventricle (0·124 and 0·076) are indicative of the condition of the heart. A repetition of this experiment gave almost similar results. In the repeat experiment (No. 4, Table IV) the heart was not fatigued so rapidly, smaller doses of adrenaline being administered. It will be noted that again there is a fall of phosphagen, 13·3 p.c. in the auricles and 16·6 p.c. in the ventricles, with an increase in the orthophosphate

TABLE V. Isolated heart-lung experiments. Phosphate fractions in Table IV

A. The effect of driving the heart of the dog with adrenaline in the presence of insulin. Periods = 30 min.

Exp. 3. Wt. of heart = 70 g.		(1)	(2)
Heart rate beats per min.		180	140
Blood pressure mm. Hg		120	110
CO_2 c.c.		298	416
O_2 c.c.		317	343
R.Q.		0·94	0·82
Blood sugar mg. p.c.	41	27	18
Blood NH_3 mg. p.c.	0·272	—	0·272
Blood NH_2N mg. p.c.	7·10	—	8·65

Heart glycogen = 0·311 g. p.c.
Heart NH_2N = 102·4 mg. p.c.

Remarks: Insulin 5 units/kg. to the dog from which the blood was taken, and 5 units at the beginning of each period. Adrenaline, 1 c.c., 1:10,000/15 min. Heart taken at the beginning of the third period.

Exp. 4. Wt. of heart = 67 g.		(1)	(2)	(3)
Heart rate beats per min.		204	204	200
Blood pressure mm. Hg		185	180	165
CO_2 c.c.		282	242	271
O_2 c.c.		308	317	271
R.Q.		0·91	0·76	0·69
Blood sugar mg. p.c.	34	30	23	19
Blood NH_3 mg. p.c.	0·134	0·200	0·102	0·068
Blood NH_2N mg. p.c.	8·86	—	—	8·86

Heart glycogen = 0·075 g. p.c.
Heart NH_2N = 97·45 mg. p.c.

Remarks: Insulin 5 units/kg. to the dog from which the blood was taken, and 5 units in each period. Adrenaline, 1 c.c., 1 : 10,000/15 min. Excellent heart, regular and strong beat throughout. Heart taken at the end of the third period.

TABLE V (*cont.*)

B. The effect of driving the heart of the dog with adrenaline in the presence of iodoacetic acid (5 c.c. = 0·025 g.)

Exp. 6. Wt. of heart = 50 g.		(1)	(2)	(3)
Heart rate beats per min.		190	180	170
Blood pressure mm. Hg		110	100	85
CO_2 c.c.		232	207	—
O_2 c.c.		230	259	—
R.Q.		1·0	0·80	—
Blood sugar mg. p.c.	81	72	47	47
Blood NH_3 mg. p.c.	0·136	—	—	0·136
Blood NH_2N mg. p.c.	9·58	—	—	10·82
Heart glycogen = 0·097 g. p.c.				
Heart NH_2N = 108·3 mg. p.c.				
Blood volume c.c.		280	250	230
Conc. I.A.A.		—	1 : 8000	1 : 4000

Remarks: No insulin given. Adrenaline, 1 c.c., 1 : 10,000/15 min. Heart collapsed in the third period; marked rigor and congestion, œdema of lungs. O_2 used in first 15 min. of third period = 70 c.c.

Exp. 7. Wt. of heart = 75 g.		(1)	(2)	(3)
Heart rate, beats per min.		120	110	90
Blood pressure, mm. Hg		90	87	80
CO_2 c.c.		250	232	192
O_2 c.c.		255	260	260
R.Q.		0·98	0·89	0·74
Blood sugar mg. p.c.	96	84	—	58
Blood NH_3 mg. p.c.	0·200	—	—	0·135
Blood NH_2N mg. p.c.	9·41	—	—	8·87
Heart glycogen = 0·061 g. p.c.				
Heart NH_2N = 95·05 mg. p.c.				
Blood volume c.c.		320	300	260
Conc. I.A.A.		—	1 : 18,000	1 : 16,000

Remarks: No insulin given. Adrenaline, 0·5 c.c., 1 : 10,000/15 min. Heart regular and in good condition; showed no signs of failure. No marked œdema of the lungs. Heart developed rigor quickly.

Exp. 8. Wt. of heart = 62 g.		(1)	(2)	(3)	(4)
Heart rate, beats per min.		144	144	120	100
Blood pressure, mm. Hg		90	95	90	80
CO_2 c.c.		245	193	161	140
O_2 c.c.		208	208	190	187
R.Q.		1·2	0·93	0·81	0·75
Blood sugar mg. p.c.	91	82	—	—	64
Blood NH_3 mg. p.c.	0·272	—	—	—	0·068
Blood NH_2N mg. p.c.	8·22	—	—	—	8·64
Heart glycogen = 0·102 g. p.c.					
Heart NH_2N = 101·7 mg. p.c.					
Blood volume c.c.		530	500	470	450
Conc. I.A.A.		—	1 : 20,000	1 : 18,800	1 : 18,000

Remarks: No insulin given. Adrenalin, 0·5 c.c., 1 : 10,000/15 min. Heart in good condition throughout, with regular and strong beat. No signs of failure. Heart removed at the end of the fourth period. Signs of congestion; developed rigor. No marked œdema of the lungs.

in the auricles of 7·2 p.c. and in the ventricles of 84·0 p.c., and a decrease of pyrophosphate amounting to 22·8 p.c. in the auricles and 56·4 p.c. in the ventricles. In this case the ventricles have suffered, as indicated by the phosphagen index (0·098), while the auricles are still in excellent condition. In this experiment the heart was almost completely depleted of its glycogen; the respiratory quotient fell from 0·91 to 0·69; the blood sugar from 34 to 19 mg./100 c.c.; there was no change in the amino N and there was a fall in the ammonia N of the perfusing blood. These results corroborate the findings obtained from many heart-lung experiments in which the R.Q. has been determined. Exp. 5 shows how marked can be the loss in phosphagen and pyrophosphate, when the heart is exhausted by adrenaline in the presence of insulin. This heart stopped suddenly in a condition of fibrillation.

In view of these results with insulin it was natural to proceed to the investigation of the effect of iodoacetic acid when added to the perfusing blood. The results of Exps. 6, 7 and 8 shown in Tables IV and V, are all closely comparable. It must be pointed out that I.A.A. in small repeated doses will always bring a heart into rigor and that in the presence of an excellent oxygenation of the blood. In these experiments no insulin was given, the heart was made to do a considerable amount of work by the repeated addition of adrenaline. In the first experiment of this series (No. 6, Tables IV and V) the heart quickly went into rigor because of a too large dose of I.A.A. Still the results are of interest because of the apparent good condition of the auricle in the presence of a collapsing ventricle. In the following experiment (No. 7) the dose of I.A.A. was reduced with the result that only after 2 hr. did the heart begin to show any signs of failure. Failure can always be detected by the alteration in gradient of the record of oxygen consumption. The change in the oxygen line is due to a loss of elasticity and the onset of œdema in the lungs. As soon as this became evident the heart was removed. These hearts were always congested and passed into a condition of marked rigor even before they could be placed in the liquid air. This rapid onset of rigor differentiates the iodoacetic acid poisoned heart from that which has been asphyxiated by a pure oxygen lack or killed by chloroform. When I.A.A. is given in small repeated doses so that the heart can beat satisfactorily for at least 2 hr., it is found that the auricles and ventricles suffer equally, but if poisoned rapidly the failure is ventricular. Exp. 6 is an example of the latter condition. There is no loss of phosphagen from the auricles, the phosphagen index is one of the highest ever recorded in these experiments, 0·233, the orthophosphate is normal, 25·3 mg./100 g.,

but there has been a loss of 32·4 p.c. in the pyrophosphate. These findings contrast markedly with those for the ventricles. The phosphagen has fallen 23·3 p.c., the phosphagen index is 0·130, the orthophosphate has increased 20·0 p.c. and the pyrophosphate has decreased 75·2 p.c. The blood sugar fell from 81 to 47 mg./100 c.c., there was no evidence of ammonia production nor of amino-N utilization, but after the second dose of I.A.A. at the beginning of the third period when the heart failed rapidly, there was a definite fall in the oxygen utilization. Cardiac glycogen was reduced to the small figure of 0·097 g./100 g., but there was no evidence of loss of amino N from the heart muscle. In the two following experiments (Nos. 7 and 8, Tables IV and V) the reduced amount of I.A.A. given made it possible to remove the heart when there were no signs of failure in its action. In No. 7 the phosphagen loss in the auricles and ventricles was 23·3 and 30 p.c. respectively; the orthophosphate increase, 10·4 and 42·8 p.c., and the pyrophosphate decrease, 56·0 and 66·4 p.c. respectively. Compare these results with the next experiment which ran for 2 hr., the heart being in excellent condition throughout. The phosphagen loss for auricles and ventricles, 31·6 and 45·0 p.c., with indexes for each show that the reserves of energy have been definitely reduced, particularly in the ventricle. Increases of 5·6 and 46·8 p.c. in the orthophosphate fractions of the auricles and ventricles respectively stand in contrast to decreases in the pyrophosphate fractions of 56·4 and 62·0 p.c. for the respective chambers. Chloroform poisoning (Exp. 9) presents a picture which is of interest in that it shows a marked similarity between the auricles and ventricles both losing heavily in phosphagen and pyrophosphate.

In all these cases, with the exception of Exp. 9, the heart was made to do an amount of work far in excess of the normal. This resulted in a marked depletion of its glycogen store but did not bring about any definite fall in the blood sugar. This is to be expected in view of the action of iodoacetic acid on the cyclic changes in carbohydrate metabolism in muscle. In Exps. 7 and 8 the respiratory quotient is reduced from unity to 0·75. This shows clearly that the sugar in the blood has not been utilized. There is either no change or some reduction in the ammonia of the perfusing blood, in one experiment (No. 7) there is a small loss of amino N. Figures for the amino-acid nitrogen of the heart muscle would not lead one to suppose that there was any definite loss of amino N from the muscle.

Urea nitrogen. When blood perfusing the isolated heart-lung preparations is well oxygenated and almost sugar-free, there is no increase in

ammonia but there is an increase in urea. In Table VI are set out the results showing the effect of aglycæmia on the production of urea N in the excised heart of the cat and the excised heart and the isolated heart-

TABLE VI. Urea production in the excised heart of the cat and dog, and the isolated heart-lung preparation, aglycæmia

All dogs from which blood was taken were given 5 units of insulin per kg. of body weight. Blood sugar and urea N content of the perfusing blood in mg./100 c.c.

Blood sugar		Urea		
Beginning	End	Beginning	End	Remarks
		Cat		
41	10	3·59	4·58	5 units of insulin in all experiments. Perfusion = 2 hr. Vol. of blood = 200 c.c.
29	12	4·06	3·72	
30	10	4·08	4·22	
30	Nil	3·95	4·08	
26	Nil	3·64	3·82	
22	Nil	3·12	4·52	
11	Nil	3·77	5·50	
	Average	3·74	4·35	Aglycæmia increase = 0·61 mg.
		Dog		
76	34	12·58	11·65	5 units of insulin in all experiments. Perfusion = 1 hr. Vol. of blood = 200 c.c.
8·1	Nil	10·16	15·91	
6·7	Nil	8·34	9·48	
2·0	Nil	10·78	15·87	
Nil	Nil	7·69	9·04	
	Average	9·91	12·39	Aglycæmia increase = 3·31 mg.
		Isolated heart-lung		
39	17	9·10	11·00	5 units insulin every 30 min. Perfusion = 1½ hr. Vol. of blood = 600 c.c.
48	Nil	10·30	11·80	
87	Nil	8·5	9·6	
36	Nil	9·3	10·4	
	Average	9·3	10·7	Aglycæmia increase = 1·4 mg.

lung preparation of the dog. Taking into account the volume of blood in circulation the increase in urea N is 1·22 mg. for the excised cat's heart, 6·62 mg. for the excised dog's heart and 8·4 mg. for the isolated heart-lung preparation, and taking 15 and 50 g. as the average weights of the cat's and dog's hearts respectively, this gives a urea-N production in mg./100 g./hr. of 4·06 for the excised cat's heart, 13·24 for the excised dog's heart and 11·20 for the isolated heart-lung preparation.

Creatine. Table VII gives the creatine content of the perfused excised cat's heart with and without insulin, first in the absence and second in the presence of glycine. It will be noted that the average loss of creatine in the aglycæmia heart is 86·2 mg./100 g. after 2 hr. perfusion, and that this is reduced to 32·4 mg./100 g. by the addition of glycine.

TABLE VII. Creatine content of the excised cat's heart perfused with and without insulin and glycine. Amounts in mg./100 g.

Perfusion with 200 c.c. blood for 2 hr., 5 units of insulin per kg. to dogs from which blood was taken, and 5 units to perfusing fluid

Without glycine		With glycine	
Normal	Aglycaemic	Normal	Aglycaemic
231	130	210	164
225	159	212	186
211	102	185	173
217	123	226	197
203	124	204	155
146	81	Average 207·4	175·0
176	112		
230	108		
Average 203·6	117·4		
Differences 86·2 mg.		32·4 mg.	

DISCUSSION

Meyerhof & Kiessling [1935] have shown that in muscle extracts glycogen in the presence of phosphoric acid reacts with pyruvic acid to form phosphoglyceric acid and lactic acid. This reaction is inhibited by iodoacetic acid. Parnas *et al.* [1934] have shown that the iodoacetic acid poisoned ventricle can be revived by phosphoglyceric acid in anaerobic conditions. The part played by adenylic acid as a donator and acceptor of phosphorus depends upon its reaction with phosphopyruvic acid [Parnas, 1935], the adenylpyrophosphoric acid transferring the phosphorus to glycogen or glucose [Lohmann, 1932]. This transference can be carried out in the presence of iodoacetic acid, the ester formed being a hexose-monophosphate [Euler & Adler, 1935] beyond which point the usual phosphorylations cannot proceed. Evidence of this is seen in the increase of reducing substances in the blood perfusing the heart and in the marked depletion of cardiac glycogen. The initiating reaction of adenylpyrophosphoric acid with glucose, giving rise to the hexose-monophosphate, produces no energy, the further reaction, the formation of the hexose-diphosphate, is markedly exothermic (24,000 cal.). In iodoacetic acid poisoning this source of energy is not available. Unfortunately the relation between aerobic and anaerobic events in muscle contraction is not precisely known. In asphyxia the aerobic oxidation upon which glycogen resynthesis depends is destroyed, and with it the initiating reaction. The breakdown in the cycle of carbohydrate phosphorylations deprives the muscle of energy and phosphorus, with the result that phosphagen and adenylpyrophosphate are depleted. This is

seen in the perfused excised heart of the cat and dog, where asphyxia brings about a rapid initial breakdown of phosphagen followed by a loss of adenylpyrophosphate, the phosphagen loss being some 20 p.c. greater than the adenylpyrophosphate. It has been shown by Ferdmann & Feinschmidt [1936] that in resting skeletal muscle of the frog the pyrophosphate fraction is almost entirely adenylpyrophosphate, the inorganic pyrophosphate forming not more than 4 p.c. of the whole. They further demonstrated that fatigue reduced the phosphagen by 80 p.c., the adenylpyrophosphate by 50 p.c. In the isolated heart-lung preparation where the blood sugar has been reduced by insulin, and the heart worked hard by the administration of adrenaline (Nos. 3 and 4, Table IV), we have obtained average percentage reductions in phosphagen of 16·6 in the auricles, 24·9 in the ventricles; in adenylpyrophosphate of 31·0 in the auricles and 50·6 in the ventricles. Exp. 5 shows the result of driving the heart aerobically to exhaustion. This heart stopped in fibrillation.

With iodoacetic acid the results are not entirely similar, since the greatest loss is in the pyrophosphate fraction and both auricle and ventricle suffer equally. The conditions of asphyxia and iodoacetic acid poisoning are not exactly parallel ones, for iodoacetic acid has an effect upon the structure of the muscle which is certainly not that of asphyxia. The reason for the great decrease in the pyrophosphate fraction as compared with the loss of phosphagen is not clear.

A consideration of the changes in the phosphorus fractions in relation to the gaseous metabolism of the heart shows that where by the action of insulin, sugar has been withdrawn from the circulation, the heart continues to beat with vigour, suffers no diminution in oxygen consumption for 1–1½ hr. and rapidly changes from carbohydrate to fat metabolism. That the aglycæmic mammalian heart could make this equicaloric change to fat for the supply of its energy needs was suggested by Cruickshank & Startup [1933*b*]. Experiments by Grimlund [1936] support the suggestion that the resynthesis of phosphagen may take place during aerobic contraction at the expense of various substances including fat, and that the formation of lactic acid is not necessary for this reaction. We find, however, that when the heart is contracting apparently sucessfully with an R.Q. of 0·70 there is a loss of phosphagen and pyrosphosphate. This is a progressive loss and is definitely in evidence in a 1½ hr. perfusion when the heart has been driven by adrenaline administration. Failure occurs suddenly and is preceded by a definite fall in oxygen utilization. Sugar will restore an exhausted heart, amino acids apparently will not,

fats have not been tried. The loss of phosphagen may indicate that fat oxidations alone are not sufficient immediately to produce the energy for resynthesis. That oxidations may not necessarily represent the effective production of energy has been suggested by Clark *et al.* [1937].

Ammonia formation. The work of Ferdmann & Feinschmidt [1936] on excised frog's hearts and skeletal muscle would indicate that in work or fatigue there is an increase in adenylic acid; that of Clark and his co-workers that there is an increase of ammonia and urea N. If adenylpyrophosphate were entirely broken down then there would be a production of ammonia and inosinic acid. Recent work has shown that by estimating the inorganic phosphates directly by means of the specific pyrophosphatase of Lohmann [Lohmann 1934*b*; Ferdmann *et al.* 1936] no inosinetriphosphate is formed during muscle activity. That the production of ammonia is a non-reversible process and that resynthesis of adenylic acid depends on the oxidative deamination of amino acids is the opinion of Parnas [1929] and Clark *et al.* [1931]. Embden [1931], on the other hand, always maintained that the production of ammonia in the fatigued excised muscle was a reversible process.

The formation of urea and utilization of glycine. The finding that in the isolated heart-lung preparation the dog's heart did not utilize amino acids, such as glycine, alanine, etc., and so prevent or retard the rapid fall of the R.Q. to 0·70, forced Cruickshank & McClure [1936] to the conclusion that the mammalian heart could not utilize amino acids for the production of energy. It would appear from the present findings that the heart muscle is capable of producing urea and, in view of the 62 p.c. restoration of creatine by the addition of glycine to the perfusing fluid, that it can utilize this amino acid. If the deamination of glycine were required to supply glucose to replace the absent or non-available carbohydrate this would result in the production of ammonia, the raising of the R.Q. to unity, an increase in oxygen utilization and CO_2 production, changes which could easily be determined in the isolated heart-lung preparation [Cruickshank & McClure, 1936]. But, if glycine were utilized mainly for the restitution of creatine, the amount of amino-acid nitrogen required would be so small as to be within the experimental error of the method for the estimation of amino N, in muscle, and the amount of glucose produced would not be sufficient to bring about any definite changes in the R.Q. and oxygen utilization. For example, if the average loss of creatine in the aglycæmic mammalian heart be taken at 50·0 mg./100 g./hr., the loss of amino nitrogen would be 16·0 mg./100 g./hr. or 8·0 mg. for a 50 g. heart. In 600 c.c. of blood

this would give a percentage content of 1·33 mg. which would constitute a difference so small as to be within the range of the experimental error for tissue estimations. The absence of ammonia in the perfusate would incline one to the belief that urea is synthesized in the heart from ammonia and that the resynthesis of protein, as seen by the action of glycine in retarding creatine loss, occurs at the expense of amino acids in the blood. The possible hydrolytic formation of urea from arginine in the heart is still a matter for investigation.

Summary

The relation between phosphagen and adenylpyrophosphate in the mammalian heart has been investigated. In the excised heart of the cat arrested at certain stages of asphyxia, phosphagen loss preceded that of adenylpyrophosphate, and in complete asphyxia the loss is approximately 80 and 60 p.c. for phosphagen and adenylpyrophosphate respectively. Asphyxial arrest of the dog's heart gives results similar to those for the cat's heart. It has been shown that the auricle suffers far less than the ventricle, the phosphagen and adenylpyrophosphate loss in the auricle being of the order of 21·1 and 22 p.c., in the ventricle 65 and 50 p.c.

Fatigue in the mammalian heart in the presence of oxygen produces no great loss of phosphagen, the figures for the auricle and ventricle being 16 and 25 p.c. respectively, while for adenylpyrophosphate they are, for the auricle 31 p.c., for the ventricle 50 p.c. In considering these figures it must be remembered that in a normal well oxygenated perfusion of the heart lasting for 2 hr. the phosphagen loss in the ventricle may amount to 10 p.c., that of adenylpyrophosphate to 25 p.c. Fatigue, if extreme, produces a relative oxygen lack which is essentially the result of asphyxia where, on account of the breakdown of phosphagen and adenylpyrophosphate, there is a failure in the effective liberation of energy.

The action of iodoacetic acid has been discussed and it has been shown that in its presence the mammalian heart resorts to fat oxidations for the supply of necessary energy. It has also been suggested that iodoacetic acid may have some specific action on the mechanism associated with the resynthesis of adenylpyrophosphate.

It has been shown that the mammalian heart, under aglycæmic conditions, can produce urea and utilize glycine.

Respiratory quotient determinations go to support the view that in the absence of carbohydrate utilization, due to insulin or iodoacetic acid, the heart muscle finally resorts to fat as a source of energy.

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MOVEMENTS IN MIDFŒTAL LIFE IN THE SHEEP EMBRYO

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In a previous paper [1936] we presented an account of the genesis of the movements concerned in respiration as they occur in fœtal sheep. These movements were followed from their onset at about the 35th–36th day until near the 49th day. After this time the movements gradually become suppressed, and difficult to elicit. This suppression of the movements concerned in respiration is but one example of a decrease which occurs at this time of the whole activity of the animal. Until the 49th–50th day this respiration rhythm and the associated movements appeared in no way to be related to the oxygen or carbonic acid content of the blood. Both the movements and the rhythm seem to be associated not with the chemical regulation of respiration, but with the essential physiological structure of the nervous system. After the 50th day, when the general suppression of activity has begun to take place, matters are quite different. Asphyxial conditions produced by temporarily arresting the placental circulation reveal again the activity of the fœtus and the rhythmic movements concerned with respiration. We have taken up our present study at this point and present an account of our experiments, which were designed to determine the factors concerned in the suppression of respiration and the further relationship between these factors and their disappearance under asphyxial conditions.

Let us consider first the character of the normal movements of the sheep fœtus from the 40th day onward. If a fœtus of the age of 40 days ± 2 be touched on the snout with a glass rod, the limbs, neck and tail all move as a part of the general extensor response which results. These movements are invariably rapid and twitch-like in character, occupying a fraction of a second. The legs give something generally

resembling a rudimentary kick, the tail a flick. Movements of this type may be referred to as "jerky". By the 50th day of gestation the response to stimulation of the face has quite altered its character. The movements of the neck, limbs and tail have lost their twitch-like character and instead have become prolonged or sustained so that the fœtus may assume a definite posture. As a result of the stimulus to the nose, the hind legs are flexed under the belly, both fore legs are rigidly extended and abducted and the limbs are maintained in these positions while an effort is made to raise the head. Then the efforts to raise the head cease, the muscles of the limbs relax. The alteration in the character of the movements from the "jerky" to the "sustained" is of course a gradual process, but the "sustained" type is seen in its most impressive form from the 48th to the 52nd day of intra-uterine life. The word "sustained" will be used to describe this type of movement.

About the 60th day of fœtal life another even more striking change in the behaviour of the fœtus presents itself. Though the change has been gradual from the 50th day onward, the full effect is not attained until about the 60th day. The fœtus has now become quite inert. Whereas stimulation of the snout with a glass rod at 40 days caused the fœtus to respond with jerky movements and at 50 days with sustained movements, at 60 days it evokes scarcely any response at all. It might almost seem that the fœtus was moribund but, indeed, this is not the case; quite the reverse. This inertness on the part of the fœtus to external stimuli is maintained and even increased as gestation proceeds, but in the very last days before birth the fœtus again becomes active. It is interesting to note that the inertness of the fœtus occurs at the time when it completely fills the amniotic sac.

In our earlier paper we pointed out that asphyxiation aroused the inert fœtus. This fact is clearly demonstrated by the events which follow the delivery of the fœtus from the uterus with an intact placental circulation. As exposure impairs the circulation through the placenta, the fœtus rouses itself as from sleep. Even without any intentional external stimulation, the movements gradually become more extensive and of the sustained type until the general reactions resemble those which characterize the behaviour of a 50-day fœtus. They are not of course quite the same because the fœtus has grown and has become more organized, but they are definitely of the sustained type.

Further deterioration of the fœtus, due presumably to failing oxygenation of the fœtal blood in the placenta, brings about a second change. The movements gradually become less and less sustained and

become jerky, resembling those of a fœtus 40 days of age. Not until this second stage has been passed through does the whole fœtus actually become moribund in the sense of being entirely unresponsive to external stimuli because the tissues have deteriorated. It would appear, therefore, that when the placental circulation is interfered with, the fœtus, crudely and in a very general way, recapitulates in the reverse order some of the behaviour patterns which it had developed. One further point is perhaps worthy of mention. Although, as we pointed out earlier [1936], occlusion of the umbilical cord does not induce activity in fœtuses of less than 50 days, it does produce activity in older fœtuses until birth. The amount of activity induced in any fœtus appears to be directly proportional to the inactivity of the fœtus before the umbilical vessels were clamped off.

Results very similar to these have already been presented by Angulo y Gonzales [1930, 1933], who found that, if the circulation through the umbilical cord of a rat fœtus was arrested, the fœtus would recapitulate the maximum degree of physiological activity it had achieved but in the reverse order. For example, fœtuses of about 18 days, that could not be aroused by tactile stimulation of the hand while the circulation was intact, responded spontaneously with waving of the hand shortly after ligation of the umbilical cord. These discrete movements were followed by total mass movements. From these and similar experiments, Angulo y Gonzales formulated the following law: "Endogenous stimulations affect directly the motor centres and act first on the most recently developed units." This order of the response Angulo y Gonzales suggested was due to the fact that the new growing mechanisms have the highest physiologic gradient and are, therefore, affected first by the relative increase of metabolites or any stimulating substance in the blood. Tracy [1926] had earlier postulated that in the developing toad-fish, the accumulation of carbon dioxide in the blood might be great enough to stimulate anterior horn cells directly before they respond to stimuli *via* the sensory nerves.

Similar to our own observations and to those of Angulo y Gonzales are the experiments of Graham Brown. Graham Brown [1915] studied the activities of the central nervous system of fœtal kittens between 8 and 9 cm. long. After the kittens had been shelled out of the uterus into warm physiological saline solution, unmistakable movements of progression were obtained on producing asphyxia by pressure upon the umbilical cord, though the movements sometimes arose spontaneously. The mechanism for co-ordinate progression was shown to develop during intra-uterine life and the experiments demonstrated that the

co-ordination of the mechanism was not conditioned by learning. Graham Brown further concluded from his studies that rhythmic activity may be evoked by the general stimulus of asphyxiation before it has been evoked or conditioned by any rhythmic self-generated stimuli. A further suggestion was made that the blood stimulus was the most primitive type and that metabolites excited the motor neurones of the cord just as they did the efferent neurones of the respiratory centre. Before leaving these experiments it is interesting to note that stimulation of the superior colliculi in these animals was followed by the response seen in the adult, i.e. stimulation over the superior colliculus caused flexion of the limbs of the same side and extension of the opposite limbs. Bilateral stimulation caused extension of all four limbs. Reference will be made to this important point later.

Our own experiments, involving as they have a systematic study of fœtuses from the 20th day following insemination onward, indicated that in the sheep at least, contrary to the conclusions of Graham Brown for the kitten and the observations of Angulo y Gonzales on the rat, the efferent neurones of the spinal cord and those of the respiratory centre could be excited *via* afferent nerves about 2 weeks before any response could be elicited by occlusion of the fœtal side of the placental circulation. Inasmuch as the responses which were elicited by asphyxiation were similar to those we had seen earlier in the developmental history but which had disappeared, we began to wonder whether the change in behaviour due to asphyxiation of the fœtus was not due to a release from inhibition rather than to direct excitation of any neurones. This release from inhibition might be caused by the death or depression by asphyxiation of the cells responsible. A parallel case in adult human physiology might be drawn with the change in the Babinski sign in man during Cheyne Stokes breathing. During the periods of apnea when the oxygen content of the blood presumably falls, the plantar stimulation is followed by an extension and fanning of the toes indicating that the influence of the cortico-spinal tract has been removed from the cord and the primitive reflex pattern released [Tournay, 1927].

If asphyxiation influences behaviour by rendering inactive inhibitory centres operating on the spinal cord and medulla, it should be possible to produce similar results by transecting the brain stem cephalad to the medulla.

This postulate was supported by the findings of Minkowski [1923] with regard to the development of the plantar reflexes in human fœtuses. For example, stimulation of the plantar surface of the foot of a fœtus a

little over 2 months old is followed by plantar flexion of the foot, while in a 4–6 months old fœtus dorsiflexion occurs. Upon prolonged exposure in the absence of adequate placental circulation, stimulation of the plantar surface of the foot of fœtuses between 4 and 6 months old is no longer followed by dorsiflexion but by plantar flexion. This same reversal in the response of these fœtuses was obtained after section of the spinal cord in the cervical region and in some cases of the brain stem just between the pons and the mesencephalon. In this particular reflex, therefore, similar results were obtained by asphyxiation and by transection of the central nervous system.

To test our postulate experimentally, in the season of 1934–5 we made some acute experiments, but we found the results difficult to interpret as the release, if any, was combined with the shock and possible stimulation which resulted from the transection. These attempts demonstrated that it was essential to produce chronic fœtal preparations. This would necessitate delivering the uterus of the mother under sterile conditions, reaching the fœtus and making an accurately placed lesion in the brain stem or spinal cord. How long the fœtus should remain in the uterine environment to recover from the operation before it could be examined, we did not know. We were encouraged to attempt these experiments by the fact that such intra-uterine operations had been successfully carried out on rats by Nicholas [1925] (see also Hooker & Nicholas [1930]) and on rabbits by Bohr [1925]. Nicholas succeeded in transecting the spinal cord and recovering the young when they were born alive at the end of a normal gestation period. So far as we are aware, however, no such operations have been reported on animals whose young are born with a relatively mature nervous system. Though the choice of the sheep for these operations was determined by other factors, it was very fortunate for the cotyledonary placenta allows the fœtus to be easily approached. Our approach was varied in the first few operations and we finally evolved the method outlined below as the one most likely to produce a successful result.

The ewe was prepared for the operation by first clipping her abdomen free from wool. The operation site was then shaved and scrubbed clean with bichloride of mercury soap. The skin was next treated with ether and swabbed with alcohol and finally tincture of iodine. A scratch was then made with a scalpel along the proposed site of the incision. The blood which oozed from this wound stained the sterile gauze laid over the abdomen and served to indicate the line of the incision after the ewe was draped.

Under ether the abdomen was opened by a mid-line incision and the uterus was delivered. Especial care was taken to avoid tension on the pelvic viscera. We found it necessary to deliver the uterus as completely as possible, and, to prevent its cooling, we wrapped it immediately in towels soaked in sterile Ringer's solution at about 45° C. These towels were frequently changed during the course of the operation. In addition, we suspended an electric heater over the operation field so that its rays served to warm the exposed viscera.

Fœtuses between the ages of 40 and 45 days old were not easily located in the intact uterus even after it had been delivered. In the early operations we made a series of small wounds along the surface of the uterus until the fœtus was sighted. These wounds were then all closed up except the one through which the operation on the fœtus was finally performed. We gave this method up, however, when we discovered that the uterus could be transilluminated with a long slender frosted electric light, which gave off very little heat. This bulb was sterilized by keeping it in a solution of bichloride of mercury when not in use. With no overhead lighting and this bulb lighted under the uterus, the fœtus could be easily distinguished and its orientation determined. The method of transillumination also had the advantage that it displayed clearly the vascular pattern of the uterus; hence it was possible to incise the musculature of the uterus with the loss of only a few drops of blood. Great care was taken that this first incision should do no more than separate the muscular coat. The chorion was then dissected away from the surface of the amniotic sac, leaving the sac intact. The incision in the musculature ran transversely to the axis of the uterine horn and was about an inch in length, each end being limited with a single gut suture. The transillumination was continued and with a finger inserted through the opening in the uterine wall the fœtus was manipulated so that the site of the proposed operation upon it was directly under the uterine wound. The surface of the fœtus could then be clearly seen through the still intact amnion. Gut sutures were next passed through the uterus just lateral to the incision and then through the musculature of the fœtus. These when completed and tied on either side served to anchor the fœtus in place. Using the uterine incision as a window, a cataract knife could be inserted through the wall of the uterus into the amniotic sac and used to transect the spinal cord or the medulla without any loss of fluid. In older fœtuses of 70–90 days we found it necessary to open the amnion and perform the operation through the uterine window. If the fœtus were held tightly against the wall of the uterus, only a few c.c. of fluid escaped. We were

not always so fortunate but the loss of 50–60 c.c. of fluid did not prejudice the outcome of the operation, except to limit the space in which the fœtus developed. In these cases the limbs were usually mis-shapen. The amniotic fluid once lost did not appear to be replaced.

After the operation on the fœtus was completed, the wound in the uterus was closed with gut sutures, and those which had served to fix the fœtus during the operation were removed, so that the fœtus was again free in the amniotic sac. No attempt was made to repair the amnion in those cases in which it had been incised. Despite this fact, it repaired itself without adhering to the wall of the uterus. The uterus was then returned to the abdomen and the body wall repaired by a series of interrupted sutures. The skin was closed with metal clips and the wound dressed with iodine and collodion.

Twenty ewes and fœtuses were operated upon. Of these twenty, eleven fœtuses and ewes survived the operation. They were sacrificed at intervals between 1 and 13 days post operatively (see Table I and Fig. 6). In one of these, however, the lesion was not in the place intended. None of the ewes aborted its fœtus following the operation. The nine unsuccessful cases did not appear to be directly due to the operation itself but to the fact that ether was used as the anæsthetic. Simpson & King [1911] called attention to the fact that sheep do not tolerate ether well and we are able to confirm their statement. In those cases —and most of them were among our first few operations—in which the time under the anæsthetic exceeded 30 min. or a total of more than 80 c.c. of ether was administered, the outcome was always unsuccessful. After the first 24 hr. these ewes began to breathe very heavily. This heavy breathing continued for several days and usually culminated in the death of the ewe. In those cases in which the ewe survived, the fœtus was always dead. It seems probable that the mortality could be materially reduced by the use of a spinal anæsthetic such as duricaine. For operations of shorter duration ether was satisfactory.

When the fœtus was to be delivered for the final observations, the ewe was anæsthetized with duricaine and placed in a bath of warm saline. Under this saline the uterus was again delivered. The fœtus was usually observed first in the intact amniotic sac and later in the saline bath. Besides our notes a cinematographic record was usually taken of the entire experiment from the moment the uterus was delivered until the end. The fœtuses were fixed in formalin after death. The entire brain stem and spinal cord was exposed by dissection to ensure adequate fixation. In some instances it was possible to check the site of the lesion

by gross dissection alone, particularly the lesions of the spinal cord. In the other cases the brains were embedded in paraffin and sectioned serially. Since the fœtuses were not old enough to possess myelinated fibre tracts, we did not attempt Marchi preparations but stained the sections with Delafield's hæmatoxylin.

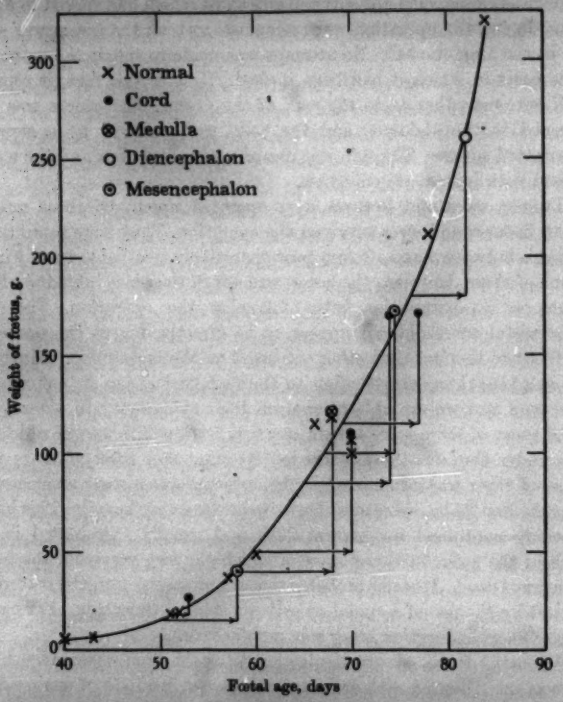

Fig. 1. Continuous line represents normal growth rate curve of sheep's fœtus. × = un-operated control. Circle = fœtuses sectioned in the situations indicated. Horizontal arrow leads from day of operation, and the circle and its contents at top of vertical line indicate day of examination and location of lesion.

Before being fixed in formalin each fœtus was weighed. These weights are indicated in Fig. 1. In each case the weights and lengths of the fœtuses at the time they were examined were well within the average for

normal fœtuses of the same age in days, so we may infer that the growth of the fœtuses was little if at all impaired by the operative interference.

That the results presented below and ascribed to the lesions in the central nervous system are not due to the other aspects of the operation is clearly indicated by the following accidental control. The lowest transection of the spinal cord which was attempted was in the region of the brachial segments (sheep 205). This experiment failed of its purpose, but formed an excellent control. The section was attempted on the 66th day and the examination was carried out on the 72nd day of fœtal life. The fœtus appeared to be in all respects a normal fœtus for its age including the fact that when the umbilical cord was compressed respiratory movements were induced. On post-mortem examination it turned out that the instrument used to produce the lesion had missed the spinal cord itself, which was untouched. It appears therefore that our general experimental procedure did not prejudice the fœtus.

The effect of lesions of the brain stem and spinal cord on the activity of fœtuses between 60 *and* 80 *days old*

Ten days before the fœtus of sheep 176 was examined, an attempt had been made to transect its spinal cord in the upper cervical region. At autopsy, the transection was found to be complete at a level between the 3rd and 4th cervical segments. When the uterus was first delivered, the fœtus inside could be seen moving very actively in contrast to the inertness of a normal fœtus of that age. The fœtus was next delivered in the intact amnion. When the sac was shaken the extremities moved, but the head did not, nor were there any attempts at righting movements. When the fœtus was removed from the amniotic sac, it became obvious that the head and upper neck muscles, like those of a normal 70-day fœtus, were quite inactive, whereas the limbs were continually executing jerky movements. Examination of the film record of the experiment showed that at first stimulating the mouth with a glass rod produced little effect, but later when stimulated in the same way, the lip and tongue moved and the nostrils wrinkled. Stimulation of the nostril with the rod once produced contraction of the orbicularis oculi, otherwise only movements of the nostril. Stimulation of the nose never produced head movements. After the fœtus had been exposed for a short time, stroking the angle of the mouth and the region of the lower jaw was followed by opening of the mouth. When an instrument was introduced into the mouth, the tongue curled as in sucking. All of these movements, which

appeared only after the fœtus had been exposed for some time, were of a deliberate and slow character.

On the other hand, from the start the fore and hind legs were actively moving as well as the tail. These movements were all of the "jerky" type and at no time did the limbs assume any sustained positions. In contrast to the inertness of a normal fœtus of 70 days, flexion reflexes with after discharges were present in both fore and hind legs. These reactions could be elicited by merely touching the dorsal surfaces of the feet, whereas only feeble flexion reflexes can be elicited in a normal fœtus when the feet are severely stimulated. Crossed extension could be produced by lightly pinching the phalanges of either the fore or hind limbs. The scratch reflex could be elicited from an area of skin over the shoulder and back. This area seemed to one of us (Barcroft) to be less well defined at the edges than the corresponding area in a dog once shown him by Sherrington. The type of movement of the leg in the scratch reflex, however, was characteristically that of a 40-day fœtus. These results are of interest for we have never been able to elicit a scratch reflex in a normal 70-day fœtus.

The above results, supported by similar information from the fœtuses from sheep 182, 196 and 211, seem to show that the mechanism responsible for the jerky type of body and limb movements is proper to the spinal cord, but that in normal fœtuses the "jerky", almost continuous activity, together with the hypersensitivity, is overruled and inhibited by influences which come from higher up in the brain stem. Our operation appeared to have released the cord from these influences. These higher centres appear to be responsible for the sustained movements. One other fact is of interest. Asphyxiation did not alter the character of the movements caudal to the transection though it did release the inhibition on those cranial nerves which were still connected with the brain stem.

The question arises: How far forward up the brain stem must we go with our transection in order to be above the seat of these inhibiting influences? To answer this question we made a series of sections at different levels of the brain stem and then studied the behaviour of these fœtuses as compared with normal and with spinal fœtuses. In the fœtus of sheep 212 the brain stem was completely transected just posterior to the pons. This was the most active of the fœtuses we examined. The legs were continually flexing and extending but not in a co-ordinated fashion one with the other. Most striking was the hypersensitivity of the fœtus when compared with a normal or a spinal fœtus. Merely touching

the dorsum of the fore foot was followed by a lively flexion, crossed extension and a generalized body reaction. This was equally true of the hind legs. However, when the section lay anterior to the pons, as in the fœtus of sheep 208, this hypersensitiveness was quite absent. The limb movements were of the "jerky" unsustained type but they were definitely more difficult to elicit than in those fœtuses in which the section was lower. Asphyxiation, in this case, did serve to increase the responsiveness of the limbs to stimuli applied to them. Sustained movements were only observed in those fœtuses in which the level of the section lay anterior to the level of the red nucleus as in the fœtus of sheep 235. The type of limb movements therefore did not alter fundamentally until the section encroached upon the diencephalon. (We use the word "fundamentally" advisedly, for close examination of the films suggests that the above description is only a rough one and that actually modifications of a minor character take place lower down the brain stem.) It cannot be affirmed with confidence that we ever got any limb movement which was not of the jerky unsustained type in fœtuses in which the brain was severed from the spinal cord. There were, however, signs of sustained movements in fœtuses whose brain stems were sectioned anterior to the pons, while in one sectioned through the superior corpora quadrigemina the movements were definitely less jerky than in the "spinal" fœtus, though they could not be called sustained. It seems probable that the factor which inhibits the "jerky" movements is present to an increasing degree as we ascend the brain stem, but its development is not marked until the mid-brain is reached and only becomes dominant when we have passed above the superior corpora quadrigemina. The highest section we made was that referred to as being above the red nucleus (sheep 235). Evidently the majority of the cells responsible for the inhibition of the sustained movements were above this level.

Having determined that a release similar to that produced by asphyxiation may also be brought about by removal of the influence of higher centres on the spinal cord, it is natural to ask what cells and fibres are responsible for the inhibition of the activity of the spinal cord. The answer to this important question is not easily obtained, for, as we have stated earlier, medullation has not proceeded far enough in the fœtuses studied to enable one to use the Marchi technique. The answer can, however, be inferred in part. Having once established the level and the extent of the lesions by examination of the serial sections of the central nervous systems stained with Delafield's hæmatoxylin, we may

infer which fibre tracts were severed by comparing these brains and cords with controls stained with one of the standard silver techniques.

A detailed account of the development of the brain of the sheep is in preparation, but it is sufficient to state here that, of the descending fibre tracts running to the spinal cord, the following appear to have reached their final destination in the 52-day-old fœtus (80 mm. crown-rump length): the cortico-spinal, the rubro-spinal, the vestibulo-spinal and the reticulo-spinal. The integrity and functional activity of the reticulo-spinal system seems evident from the fact that respiratory movements are executed by the diaphragm. The typical responses assumed to be due to impulse discharges descending *via* the rubro-spinal tracts may be elicited by electrical stimulation of the superior colliculi as early as the 45th day of gestation. The fibres of the cortico-spinal system can be seen decussating in the 52-day fœtus and even in the adult sheep the fibres of this tract have not been definitely traced further into the cord. The motor cortex is, however, not excitable in fœtuses of the ages used in these experiments. We have no evidence to indicate whether or not the vestibulo-spinal tract is functional in these animals though it may be clearly distinguished in very young embryos.

Though these inferences must be tentative, it would appear that in the absence of all these descending tracts the activity of the spinal cord in the fœtuses studied manifests itself in jerky unsustained movements. With a part at least of the reticulo-spinal system projected into the cord, this activity seems to be enhanced. The first system of fibres which appears to inhibit activity in the spinal cord is the vestibulo-spinal, for in those animals in which the vestibular nuclei were intact the activity of the fœtus was markedly reduced. The final stage of inhibition of the jerky movements and the presence of sustained movements appear to depend upon the integrity of the red nucleus and the rubro-spinal system. Inhibition of these sustained movements appears to be due to structures lying anterior to the mesencephalon and the red nucleus.

These results appear, therefore, to support the hypothesis that the gradual inhibition of activity in the fœtus as gestation proceeds is brought about by the fibre systems which descend into the spinal cord. In the sheep, as Minkowski [1923] has shown to be the case in the human fœtus, the tegmentum appears to be responsible for the first stage of the inhibition. The further degrees of inhibition which appear later in the developmental history are provided by centres lying farther forward in the fore brain. They further support our suggestion that asphyxiation increases the activity of a dormant fœtus by the removal of these

inhibiting influences and not by direct excitation of any efferent elements. Our results give no support to the thesis that CO_2 accumulation can excite any cells directly though they do not rule it out, but indicate that asphyxiation decreases and inhibits the activity of the cells of the central nervous system. The recapitulation of the behaviour patterns in their reverse order as described by Angulo y Gonzales, Minkowski and ourselves demonstrates that there is a susceptibility gradient to asphyxiation laid down in the brain stem as it develops. The susceptibility seems to be highest in the more cephalic parts of the brain stem. Hence asphyxiation seems to release the cells of the medulla, including the postulated respiratory centre, from higher inhibiting centres. Thus released, the respiratory pattern appears in the pattern in which it was present in the 48-day fœtus. The fundamental point seems to be that, contrary to common belief, the cells of the respiratory centre are not the most susceptible to asphyxiation. These considerations present the action of asphyxia on respiration in a somewhat different light to that in which it is frequently viewed. The conception of asphyxia as *stimulating* the respiratory centre appears to be at least partially untrue. Asphyxia obviously stimulates the centre in the sense that it causes an increase of the total ventilation, but does it stimulate the cells of which the respiratory centre is composed? Of this our results furnish no evidence either way, though they do demonstrate that great activity of respiration results from release of the medullary centre from the influences that control it. The most obvious effect of asphyxia (that combination of oxygen want and CO_2 excess wrought by occlusion of the umbilical cord) is depression of these higher cells and not stimulation of the lower ones. While no cell in the body is likely to be independent of asphyxia, the inherent activity of the medullary centre seems to be regulated primarily by quite other considerations—considerations which have their origin in the rhythmic mass movement observed on the 40th day; this movement we have referred to as "automatic", but only in the sense that we have not as yet been able to discover a controlling cause.

Respiratory movement

In the above description of fœtal movements we have confined ourselves to the movements of the limbs, apart from occasional allusion to those of the head. We have purposely avoided more than incidental references to the respiratory movements which must now be treated specifically.

In a former paper it was pointed out that the typical movements of respiration have their origin in a rhythm which at first involves the muscles of the body generally, but which afterwards becomes restricted to the muscles proper to respiration. This rhythm is not continuous. It can be initiated by stimulation of the fœtus and is maintained for anything up to about 20 beats.

At first all the beats are the same, but, about the 40th day, following upon stimulation of the nose the first beat or first two beats may be different from the succeeding ones in that the head is thrown back, i.e. the neck is extended. The neck movement in these beats is, therefore, one of extension followed by flexion, instead of merely flexion. In the subsequent beats the neck is flexed only. As time goes on, this differentiation between the immediate response to stimulation and the subsequent rhythm becomes more marked. The immediate response not only retains its extensor character, but it becomes "sustained" and purposive in character, whilst the subsequent movements retain their rhythmic nature and are restricted to the muscles ultimately responsible for respiration. In this connexion the "muscles ultimately responsible for respiration" is used rather in a physiological sense than as representing an anatomical group. Respiration according as it is quiet or forced involves fewer or more muscles, and the same is true in the case of which we are speaking.

By the 50th day the response to stimulation is one almost of a definite effort on the part of the fœtus to get up, followed by a respiratory rhythm which gradually dies away. The rhythmic movements of the respiratory muscles, subsequent to this initial effort, bear the appearance of an after-discharge. The appearance is as though a spring had suddenly been released and after the first recoil it had oscillated till the oscillation died away, or as though a stone had been thrown into the water and after the first splash waves had radiated from the disturbance.

Two points should be emphasized:

(1) At 50 days so delicate is the mechanism that only complete and prolonged absence of movement allows of the total abolition of the secondary rhythm.

(2) The rhythm, unlike the analogies given above, varies in frequency as well as in amplitude. If when the fœtus is quiet, or nearly so, it be stimulated, the rhythm starts as a deep and rapid one and as it dies away becomes slower and shallower. This is shown in the following record (Fig. 2) in which the rhythm was followed by a hand key. As each apparent movement took place the key was pressed down and the signal

depressed. The record shows only the frequency but not the amplitude of the rhythm. Up to this stage, occlusion of the cord elicits no respiratory rhythm whilst the cord is actually occluded though occasionally a rhythm appears when the cord is released.

Apart from the inhibition which has been already considered, respiration by the 70th day has undergone some remarkable changes. Its relation to somatic movement, if no less intimate than in the 50-day fœtus, is much less obvious. In the 50-day fœtus the rhythm, in so far as it was kept going, was kept going by muscular movements from which the rhythm followed apparently as an after-discharge. In trying to get rid of rhythmic movement the operator goes on the principle: no muscular movement, no after-discharge. In a 70-day fœtus there is by no means

Fig. 2. Record of respiratory rate of 51-day fœtus (sheep 180). Respirations almost continuous; rate increased from 4 in 11 sec. to about 4 in 5 sec.

necessarily any considerable after-discharge following a sustained movement. On the other hand, the obvious way to produce a respiratory rhythm is to occlude the umbilical cord. Respiration and movement have become largely dissociated from one another. Obviously this degree of dissociation had to come sometime, for the born animal breathes in his sleep when he makes no muscular effort except that of his own respiratory muscles, whilst on the other hand, if in good health, he makes quite considerable movements without obvious dyspnœa. On immediate exposure of the fœtus we see a clear picture: hit the fœtus on the nose or rump and it makes a sustained movement; pinch the cord and it makes respiratory movements but not somatic ones.

Yet this is not the whole story: if the fœtus be exposed for a little time the clear line of demarcation between the rhythmic and the somatic movements becomes less clear. The fœtus on little or no provocation may be seen to give very faint rhythmic movements of the diaphragm, and on hitting him these become faster and stronger. As an example, in sheep 153, fœtus 78 days old, the cord was occluded on two occasions. Reckoning

the time from the commencement of the first occlusion, the following are the relevant data:

Commencement of first occlusion	Zero
First "breath"	16 sec.
End of first occlusion	62 „
Commencement of second occlusion	103 „
First "respiration" after second occlusion ...	112 „
End of second occlusion	184 „

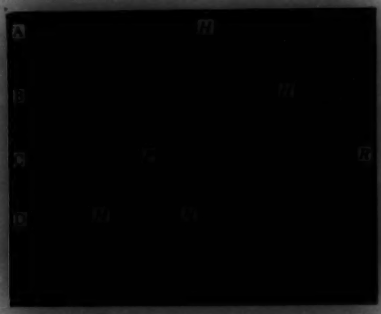

Fig. 3. Fœtus of sheep 185. A, B, C, D are parts of a single tracing. Between the end of A and the commencement of B 172 sec. have been omitted, B, C and D are continuous. The marks of the signal signify respirations. H = fœtus hit; P = cord pinched; R = cord released. Time = seconds. A, the fœtus had been exposed some time and gave shallow respiratory movements when manipulated; at H it was hit: 4 movements, a pause and then a group of movements within as many seconds. B, one hit followed by only one respiratory movement. C, manipulation of cord produced several movements and actual occlusion produced 46 movements in 41 sec. D, hitting the fœtus now evoked a much greater response than formerly.

Fig. 3 shows portions of the record of respiration rate in Exp. 185. The fœtus (70 days), after a short exposure, gave occasional respiratory movements on manipulation (A, B), and at this stage, when hit (H), gave a slight respiratory response only. On further exposure the respiratory movements became more frequent (C) and were very marked when the cord was pinched (between P and R). After pinching (D) the respiratory response to hitting was much greater than in A and B.

The general effect of asphyxia, in respect to the relation of somatic to respiratory movement, like its effect on the nature of somatic movement, is to put back the 70-day fœtus to approximately the 50-day stage. In

fact the only real difference between the two is a holding of breath for a moment while the sustained movement is taking place. This is very evident in the 70-day fœtus with the cord pinched and is less so, though we could not say it was absent, in the 50-day fœtus. The phenomenon is not without interest. We placed a heavy weight on a table at one side of a room about 15 ft. across and asked four persons successively to shift it to a table on the other side of the room. We gave them no hint as to our object. Each person held his breath while he was actually shifting the weight and his respiration was more active after he had finished the operation. The same may be noticed in many of the actions of life where a sustained and not too long effort is made.

Respiration considered with regard to spinal lesions

Having learned something of the effect of transecting the central nervous system on movement generally, let us go back to consider in greater detail its effect on respiration. Our general scheme will be to consider the lowest lesions first.

The spinal fœtus. Fœtuses 176 and 182 were sectioned between the top of the 4th cervical segment and the bottom of the medulla. In fœtus 176 sectioned on the 60th day and examined on the 70th, the section was between the 3rd and 4th cervical, and in the fœtus 182, sectioned on the 69th day and examined on the 77th, the section was approximately at the 2nd cervical. In neither case was there any sign of respiratory movement in the chest. It must be assumed then that at this age respiratory movements originate in the brain. There remains the possibility of respiratory movements in the head and neck resulting from impulses conducted along the cranial and such spinal nerves as may be above the lesion. The note on 176 runs: "Compression of the umbilical cord did not induce movements, except some of the infrahyoid region, though when the cord was released movements were each time set up in the hind legs." But reference to the film shows that the neck movements amounted to a sustained rhythm, those of the legs to a mild spasm. The note to 182 runs: "There were no sucking movements... pinching the cord does not produce any generalized convulsions. There were movements in the throat region; presumably they were associated with respiration." These movements were also rhythmic (film). There was, however, a generalized rhythm which involved opening and shutting the mouth and movements of the floor of the mouth and of the upper part of the throat.

The above observations present a side issue which we may note here but which must remain the subject for future work. What is the relation between the rhythmic throat movements in sucking, breathing and swallowing respectively? That they are related is sufficiently shown by the impossibility of breathing and drinking simultaneously. It is very difficult in our films to tell to which category a given rhythmic movement of the throat belongs. It seems possible that they all have much in common but that the nature of the afferent stimulus, including the nature of the material entering the mouth and (or) nose, gives this rhythm a bias in one direction or another. Nor is this ambiguity confined to the throat. It is true also of the mouth. It was noted (sheep 101, 49 days) that "when respiring heavily the mouth opened and when respiring more easily the mouth remained shut". The difference between these respiratory movements and chewing movements is not well defined. In this same fœtus, when a large needle was introduced into the mouth the fœtus sucked on it vigorously eight or ten times, "and when a glass rod was introduced into the mouth the lips grasped it and the jaws closed forcibly".

The medullary fœtus. Sheep 212, C.N.S. sectioned on the 80th day, examined on the 93rd. Post-mortem findings: "Lesion runs posterior to cerebellum and pons. Upper part at the level of 7th and 8th cranial nerves. Section is complete on left side and looks as if complete on the right. Respiratory movements were clearly seen even before the sac was opened. At the time of delivery and for some time after, the fœtus was respiring almost continually." Each respiration consisted of a rather jerky inspiratory movement and it was not clear that there was any active expiratory movement. The fœtus did not suck, presumably because the receptors from the 5th nerve had been severed from the lower portion of the medulla.

Section just above the pons. Sheep 208 was sectioned on the 58th day and examined on the 68th. The lesion was just in front of the pons. Respiration was almost continuous, but was more pronounced near the end of the experiment. The effect of striking the fœtus either on the head or rump did not increase the frequency of the rhythm (Fig. 4). In this the fœtus differed from 185, shown in Fig. 3, which was within 2 days of the same age. The principal results of the comparison were that fœtus 185, unless asphyxiated, gave only an occasional breath; when asphyxiated it breathed; when struck its respiration was first inhibited and then augmented. Fœtus 208 breathed actively from the start, more actively as time went on and its respiratory movements were uninfluenced by its

being struck. Presumably therefore the arcs responsible for the effect of stimulation on respiration had been severed and that involved portions of the brain above the pons.

Fig. 4. Records of respiration (*P* and *R*) of fœtus of sheep 208. *P*, shortly after exposure; *R*, after a further interval of some minutes. *H* = fœtus struck, position unspecified (nose or rump); *A* = fœtus struck on rump; *B* = fœtus struck on nose. Time = seconds.

Fig. 5. Three successive records, A, B and C, of the effect on the respiratory movements of hitting the fœtus (sheep 200). The upper signal in each case denotes the hit, the lower one the respiratory response. Time = seconds.

Section at level of superior corpora quadrigemina. Sheep 216, sectioned on 67th day and examined on 77th. Ventrally, the section was through the optic chiasma. The respiratory movements showed the same general characters as the previous one.

Section above red nucleus. Sheep 200, C.N.S. sectioned on 74th day, examined on 80th day. Post-mortem: there was no clot about the medulla or mesencephalon. The hemispheres themselves were not soft. There were two fissures in the brain.

As already stated, this fœtus differed from others in that its movements were of a tonic character. From the standpoint of respiration it

was active; inspiration was not, however, a single jerky movement but often consisted of three or four lesser inspirations, generally distinct but sometimes fused. This phenomenon disappeared when the limb movements ceased to be sustained and became jerky and was replaced by

Sheep no.	Age in days at operation	Survival in days	Age in days at final experiment	Approximate site of lesion
182	69	8	77	Between C 2 and C 3
176	60	10	70	Between C 3 and C 4
196	73	1	74	C 1
211	57	5	62	C 1
208	58	10	68	Anterior to the pons
234	51	3	54	Region of left inferior calliculus destroyed
216	67	10	77	Anterior to superior corpora quadrigemina
200	74	6	80	Through corpus striatum
205	66	6	72	Lesion attempted in cervical region but unsuccessful
235	50	8	58	Anterior to mesencephalon
212	80	13	93	Just posterior to pons

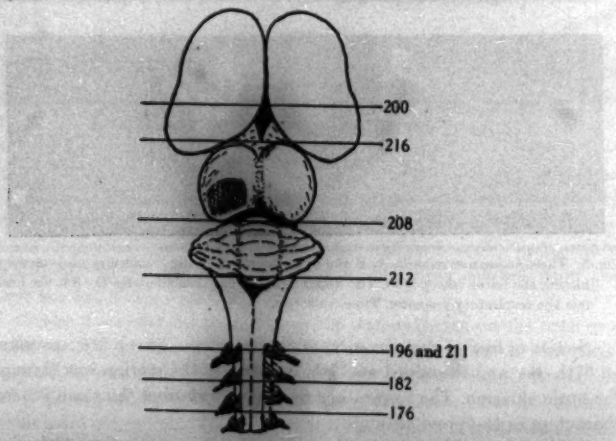

Fig. 6. Diagram of central nervous system showing approximate position of lesions. The cross-hatched area indicates the site of the lesion in No. 234. The lesion was unilateral.

gasps as seen in fœtuses sectioned at lower levels. Striking the fœtus, unlike 208, produced a hold-up in the respiration followed by dyspnœa, as may be seen from Fig. 5. Here again the respiratory movements for a

while seemed to get more active as time went on. The linkage between muscular movement and dyspnœa appears to be at the level of the red nucleus. At this stage it would be natural to institute some comparison between our results and those of Trevan & Boock [1922] and Lumsden [1923] respectively. We have found this to be impossible probably because we have no real bases for such a comparison. The following facts must be borne in mind:

(1) The vagi in our experiments have not been cut, and cutting the vagi predisposes to apneuses.

(2) Our animals, unlike those of Lumsden, were not under an anæsthetic.

(3) The movements of the chest in our fœtuses were very limited as compared with similar movements in cats with open lungs. The consequent reflexes therefore were presumably weaker in the fœtuses than in the cats.

(4) None of our sections went through the precise region invasion of which caused the phenomenon variously described as "Vagal ataxia" by Trevan & Boock and "Apneuses" by Lumsden.

The fact, however, is that in our fœtuses we have seen no change in the type of respiration as the result of transecting the central nervous system until the section gets above the mid-brain; when the respiratory movements participate with those of other parts of the body in the change from the jerky to the sustained type. This is another way of saying that the tonic element, so important in respiration [Hess, 1930; Henderson, 1936], is dependent upon the part of the brain immediately in front of the mesencephalon.

Summary

1. The normal fœtus of the sheep shows movements of certain well-defined types at different ages, namely:

(*a*) When about 40 days old the movements are rapid and "jerky".

(*b*) When about 50 days old they are drawn out and "sustained".

(*c*) Over this age the fœtus becomes quiescent.

2. Exposure of the fœtus which has reached stage (*c*) above, associated with impairment of the placental circulation, abolishes the quiescence, producing first "sustained" movements typical of the 50-day normal fœtus. These are followed by jerky movements typical of the normal 40-day fœtus, later still by immobility and finally death.

3. It has been found possible between the 50th and 76th days in the sheep to transect the fœtal central nervous system without taking the fœtus out of the uterus.

Sections have been made at levels between the 4th cervical region of the cord and the diencephalon (inclusive).

Such sections do not impair the growth of the fœtus, for the next fortnight at least, after the section is made.

4. Fœtuses in which the section is in the cervical region exhibit movements of different types according as the muscles involved are supplied with nerves from above or from below the seat of the section—if supplied from above the section the movements are normal, if from below the section the movements are of the "jerky" type. Moreover, the parts supplied from below the section are intensely active.

5. The facts as in § 4 are true of sections through or below the mid-brain with this reservation: while the movements are of the "jerky" type in all cases, they tend to become less so the higher the section.

6. If the section is through the diencephalon the movements on exposure of the fœtus are not of the "jerky" but of the "sustained" type. In further exposure they become of the "jerky" type.

7. We interpret the above as follows:

(*a*) The fundamental movements are of the "jerky" type.

(*b*) About the 50th day these become controlled by influences arising in the region of the red nucleus from which they can be released either by asphyxia or by transection of the brain below, or involving the red nucleus.

(*c*) In older fœtuses the "sustained" movements are subject to inhibition from above, from which they in turn can be released.

8. With regard to the special case of respiratory movement:

(*a*) At about 40 days this individuates out of (or perhaps more correctly is the relic of) a generalized mass rhythmic movement. At this stage it cannot be induced by asphyxia.

(*b*) At about 50 days respiratory movements are almost continuous, being evidently consequent on even quite trivial muscular movements.

(*c*) At about 60 days respiratory movements are difficult to elicit, but can be induced by asphyxia which causes the fœtus to revert to the 50-day stage.

In the 60-day fœtus (and over) in which the C.N.S. has been cut between the mid-brain and pons (inclusive) there is almost constant respiratory rhythm of a "jerky" type. If the section is above the mid-brain, respiratory movements are also frequent but are of the tonic type. Our interpretation of the above is along the lines indicated in § 7 (above), "asphyxia" or transection releasing the lower from the upper levels.

We do not, however, rule out the possibility of asphyxia acting at times as a stimulus, though it certainly, on occasions, cuts out inhibition.

It should be noted, that by "asphyxia" we mean the combination of oxygen want and CO_2 excess caused by occlusion of the cord and that we leave open the effects of oxygen want or CO_2 excess separately, on which we have done no experiments so far as this paper is concerned.

(*d*) Somatic movement and respiratory rhythm become increasingly independent as the fœtus ages, but the connexion can be restored by asphyxia. If the C.N.S. be sectioned at or below the mid-brain in the fœtus of about 70 days the connexion is definitely broken, not so if the section is at the level of the diencephalon. We regard the linkage therefore as being in the general region of the red nucleus.

We should like to record our thanks to Dr Gotsev who gave most of the anæsthetics.

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THE "PANCREOTROPIC" ACTION OF ANTERIOR PITUITARY EXTRACTS

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Anselmino, Herold & Hoffmann [1933] have claimed that injections of a crude anterior pituitary extract into young, intact rats produce, in a few days, a marked increase in the number and size of the islets of Langerhans of the pancreas. They ascribed this increase to the action of a "pankreatrope Substanz", believed to be present in the pituitary gland. This substance not only causes an anatomical hypertrophy of the islets of Langerhans, but also stimulates the secretion of insulin by the islets, as shown by the fall of blood sugar occurring immediately after injection [Hoffmann & Anselmino, 1933]. This fall of blood sugar is accentuated in the absence of the adrenal glands [Anselmino & Hoffmann, 1936*a*]. The pancreotropic substance was found to be ultra-filterable, as it would pass through a collodion membrane at *p*H 5·2 [Anselmino & Hoffmann, 1933]. Although these authors have published further investigations on this subject [Anselmino *et al.* 1935; Anselmino & Hoffmann, 1936*b*], their original claim lacks satisfactory confirmation, and in some instances negative results have been recorded [Leyton & Jones, 1936; Elmer *et al.* 1937].

Chrzanowski & Grzycki [1937] are, so far as we are aware, the only authors claiming to have confirmed the belief of Anselmino & Hoffmann [1933] that a *p*H 5·2 ultra-filtrate of an aqueous extract of acetone dried pituitary gland can stimulate formation of islet tissue. Chrzanowski & Grzycki used commercial preparations of pituitary gland and, assessing their results on the histological appearance of sections of the pancreas, found that crude aqueous extracts were inactive, although a *p*H 5·2 ultra-filtrate in acetate buffer possessed "pancreotropic activity". These authors explain their results by the suggestion that the crude

extract contains the "contra-insulin hormone" of Lucke [1933] which masks the activity of the "pancreotropic substance", and which is removed by ultra-filtration. It should be pointed out that these findings are not in agreement with those of Anselmino *et al.* [1933] whose original "pancreotropic" preparation was a simple aqueous extract of "fresh, acetone-dried anterior lobe". Ultrafiltration was introduced in a later paper [Anselmino & Hoffmann, 1933].

Krischesky [1936], using a quantitative method of islet-tissue estimation, found that in the male rat hypophysectomy *increased* the ratio of the islet tissue to body weight to about 63 p.c. above that of control animals. When the hypophysectomized animals received four injections weekly of a crude alkaline pituitary extract, beginning on the day of operation, the ratio was only 34 p.c. greater than that of the controls. These differences in ratio were not accountable to the differences in body weight. Thus, in these experiments, a crude pituitary extract appeared partially to inhibit hypertrophy of islet tissue. On the other hand, islet hypertrophy after the administration of crude pituitary extracts has been found by Aron [1933] and Bierring [1934], while Florentin & Picard [1936*a*] find that injections of thyroxin, œstrin, posterior pituitary extract and insulin may all cause an increase in the size and number of the islets of Langerhans. By counting the number of mitoses per 100 pancreatic islets in groups of young rats, McJunkin & Roberts [1932] found that insulin treatment inhibited the normal proliferative activity of the islet cells. Other investigators have found that, as judged by the histological appearance of sections of the pancreas, insulin causes hypertrophy of the islet tissue [Schereschewsky & Morguilnitzky, 1928; Collin *et al.* 1931; Aubertin & Mollaret, 1932; Lacoste *et al.* 1936], while the thyroid gland is believed to exert a similar action [Florentin & Watrin, 1931]. Cramer & Horning [1936] have described hypertrophy of the islets of Langerhans in mice as the result of prolonged administration of œstrin. This was accompanied by morbid changes in other endocrine glands, particularly in the anterior lobe of the pituitary, in which chromophobe adenomata were found.

The conflicting results in the literature may well be due to the great variation in the size and number of the islets in different animals of the same species, which may lead to erroneous conclusions with regard to the action of an injected extract [cf. Florentin & Picard, 1936*b*; Elmer *et al.* 1937].

Experiments on the action of a diabetogenic extract of anterior pituitary gland [Young, 1936] have shown that the diabetic syndrome,

elicited in a dog by the daily administration of a given amount of anterior lobe extract, usually disappears after a few days in spite of the continued daily injection of the same amount of extract. If, however, the daily dose is suitably increased the diabetic condition reappears, only to disappear again. This phenomenon of the disappearance of the symptoms and their reappearance on increase of the daily dose can be repeated a number of times. The pancreas of a dog killed during an experiment of this kind showed unusual mitotic activity in the islet cells, and it seemed possible that the disappearance of the symptoms of diabetes occurred because of an hypertrophy of the islet tissue, followed by an increased secretion of insulin by the pancreas. The presence, in a crude pituitary extract, of a diabetogenic factor together with an antagonistic but more slowly acting "pancreotropic" substance might explain these results. It was therefore of importance to investigate Anselmino & Hoffmann's claims.

In spite of the fact that quantitative methods of islet-tissue assay have been developed [Heiberg, 1906; Ogilvie, 1935, 1937; Krischesky, 1936], in most of the investigations on the action of pituitary extracts on the pancreatic islets, so far recorded, the increase in the number and size of the islets has been assessed solely on the basis of the histological appearance of isolated or serial sections of the pancreas. Such a method is subject to grave error, owing to the lack of uniform distribution of the islet tissue throughout the pancreas and the great variation in the size of the individual islets. For this reason an objective and quantitative reinvestigation of the problem was undertaken.

Methods

Wistar-strain albino rats of either sex and of initial weight 120–150 g. were used in these experiments. They were all fed on an adequate mixed diet.

An extract of fresh ox anterior pituitary gland was prepared according to the method of Schockaert [1931] from glands brought from the slaughter house in solid carbon dioxide as soon as possible after the death of the animal. Dried gland was prepared by acetone extraction of minced fresh anterior lobe, and extracted by grinding with cold saline; the extraction was allowed to proceed for 24 hr., after which the insoluble material was spun off. The saline extracts of both fresh and dried gland were made every 2 or 3 days and were stored at 0° C. Ether and alcohol extracts were prepared by grinding the fresh tissue with excess of anhydrous sodium sulphate and extracting exhaustively in a Soxhlet apparatus. After removal of solvent, the material was emulsified in saline for injection. A crude "tethelin" was prepared by the method of Robertson [1916] and a commercial sample (H. K. Mulford, Co., Philadelphia) was also used. The volume of all the pituitary extracts was finally adjusted so that 2 c.c. were equivalent in every case to 1 g. of fresh gland. Each experimental animal received 2 c.c. of extract subcutaneously every day for periods

ranging from 3 days to 3 weeks. Control rats were injected daily with 2 c.c. of various non-specific tissue extracts.

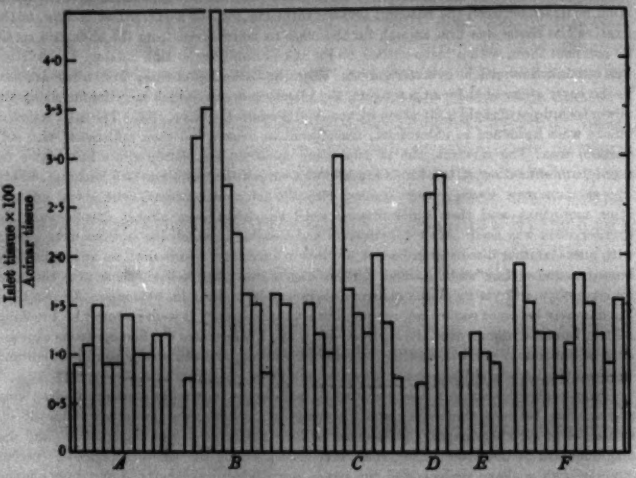
Immediately the animal had been killed the pancreas was dissected out as a single strip of tissue, intact in the mesentery, twisted into a roughly hemispherical mass, and placed on a clean slide. After the blood had clotted sufficiently for the tissue to remain attached to the slide, this was placed in Zenker-formol (Maximow) which was found to give the maximum contrast between the basophil acinar cytoplasm and the islets. After 15 min. in the fixative the tissue was firm enough for the mass to be removed from the slide and retain its compact form, with a plane surface on its base suitable for section cutting. The fixation was continued overnight in a refrigerator. With the exception of those from a few animals in the early stage of these experiments, the glands were embedded in celloidin, using the newer technique of rapid infiltration by heat and pressure [see Lee, 1937]. The solid celloidin blocks were hardened in chloroform, transferred to benzol and then infiltrated with soft paraffin wax. The sections, cut at 16μ, were mounted on photographic half-plates by a simple modification of Apathy's egg-albumin-turpentine technique [see Romeis, 1932]. The sections were strongly over-stained with Ehrlich's acid hæmatoxylin a few minutes after mounting, and then differentiated until the islets were almost decolorized. No counter-stain was used. After dehydration and clearing in xylol, the sections were coated with gum-dammar dissolved in benzol, applied in successive layers until an approximately homogeneous surface was obtained. In this way it was possible to dispense with the need for coverslips and yet to obtain sharp resolution even under $\frac{1}{4}$ in. objective. After a time the dammar becomes somewhat opaque, but its transparency is soon restored by brushing a little benzol-dammar over the surface. Pl. I, fig. 1, illustrates the homogeneous appearance of the acinar tissue following celloidin embedding, and the strong contrast obtained between the islets and the acini in a typical complete section.

From each 4th section a field, selected to include the maximum amount of islet tissue and varying between three-fifths and the whole of a section, was projected vertically on to a sheet of cartridge paper, the diameter of the circular projection being 20 cm. and the magnification 34 diameters. The circles were released from the sheets of paper by cutting directly with a sharp scalpel, and the paper corresponding with the ducts, interlobular connective tissue, fat and lymph glands eliminated in a similar manner. The islets were then outlined in pencil. The pieces of paper, representing the acinar and islet tissues together, were numbered and the odd numbers separated from the even numbers, the two piles being weighed separately. The results for thirty-six animals showed that when every 4th section of the pancreas had originally been taken, there was a difference between the weights of the odd number and even number piles of paper of less than 0·15 p.c. In the rest of the experiments every 8th, instead of every 4th section of the pancreas was therefore used. After the paper representing the combined acinar and islet tissues had been weighed, the islets were punched out, using steel punches of various diameters, with the paper placed on top of a sheet of leather. The relative weight of the islets was obtained by a second weighing.

Results

Islet/acinar ratio. The results for forty-nine injected animals, expressed as the ratio $\frac{\text{islet tissue} \times 100}{\text{acinar tissue}}$ are given diagrammatically in Text-fig. 1. There is little variation in the results for the ten control animals, the figures lying between 0·9 and 1·5 with a mean of $1{\cdot}1 \pm 0{\cdot}06$. The results for the group of eleven animals receiving a saline extract of fresh gland

for 2–3 weeks vary between 0·75 and 4·5 with a mean of $2·2 \pm 0·35$. The difference between the mean values for these two groups is $1·1 \pm 0·36$. Since this difference exceeds three times its standard error it has a high degree of significance. The mean value for the group of ten animals

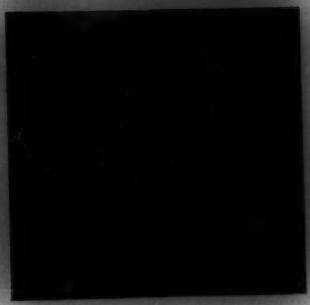


Text-fig. 1. The value $\frac{\text{islet tissue} \times 100}{\text{acinar tissue}}$ determined for rats injected with different types of extract

Group (A). Control animals.
(B). Animals receiving a saline extract of fresh anterior lobe.
(C). Animals receiving a saline extract of acetone-desiccated anterior lobe.
(D). Animals receiving a saline emulsion of the ether soluble material from fresh anterior lobe.
(E). Animals receiving a saline emulsion of the alcohol soluble material from fresh anterior lobe.
(F). Animals receiving "tethelin".

injected with a saline extract of acetone-dried gland is $1·5 \pm 0·20$. The difference between the mean value for this group and that for the control group is $0·4 \pm 0·21$, which cannot be considered as significant.

As a statistical method of analysis cannot be satisfactorily applied to the results from only a few animals, we have divided the groups which contained less than ten rats into those animals showing positive, and those showing negative results. We have considered arbitrarily that when



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the result for a given animal is equal to or greater than twice the mean value for the control group, it constitutes a positive result. On this basis two animals out of the three receiving twelve daily injections of the ether soluble material from fresh gland showed a significant increase in the islet/acinar ratio. The increase in two out of three animals injected with the ether extract, together with the relative inactivity of the acetone desiccated gland, suggested the value of further investigation of the activity of fat extracts. As samples were available of "tethelin" (a fat soluble pituitary preparation at one time believed to be the growth principle of the anterior lobe—Robertson, 1916; cf. however, Drummond & Cannan, 1922), these were tested. The results for a group of eleven animals injected daily for 1–3 weeks gave a mean value of $1{\cdot}3 \pm 0{\cdot}11$; the difference between this mean value and that for the control group is $0{\cdot}2 \pm 0{\cdot}13$ which cannot be considered as significant. Although there is no significant difference between the mean results for the control group and for the groups of animals injected with a saline extract of dried gland or with "tethelin", yet in the latter groups a number of giant islets could be observed (see Pl. I, figs. 4, 6). It is clear that the presence of giant islets is not, *per se*, satisfactory evidence of a significant increase in the total amount of islet tissue. No positive results were obtained with animals injected daily with any extract for a period of less than 2 weeks.

Estimation of acinar tissue. As the results have been expressed as the ratio of islet to acinar tissue, it was of importance to determine whether or not the observed increases in this ratio were due to a decrease in the amount of acinar tissue. Furthermore, it was of interest to know whether or not the total amount of pancreatic tissue was altered by the injections. The weight of each pancreas was not taken, owing to the variable amount of connective tissue, lymph glands and fat which cannot be removed without tedious dissection before fixation. An approximate comparison of the sizes of the pancreases can be obtained by comparing the number of sections in each series, and the amounts of acinar tissue can be compared by considering the relative weights of the paper representing acinar tissue. Judged in this way the results in Table I indicate that the injection of aqueous extracts of the anterior pituitary gland causes a slight growth in the whole pancreas, with a similar increase in the amount of acinar tissue. This increase is similar in those animals exhibiting a significant increase in islet/acinar ratio in response to an aqueous pituitary extract (B, Table I) and in those showing no significant increase in this ratio (C). As the total amount of acinar tissue increases rather

TABLE I

Extract injected	No. of animals	No. of sections per pancreas			Weight of paper equivalent to acinar tissue		
		Max.	Min.	Av.	Max.	Min.	Av.
(A) Control	10	320	224	300	205	142	180
(B) Fresh gland, saline extract	11	368	280	325	235	177	202
(C) Dried gland, saline extract	10	352	280	320	243	166	201
(D) Ether extract	3	320	280	296	186	166	178
(E) Alcohol extract	4	304	272	284	172	120	156
(F) "Tethelin"	11	336	256	292	208	131	171

than decreases in these instances, and is not significantly decreased in those animals receiving the ether extract, the recorded rises in the islet/acinar ratio must indicate an important absolute increase in the amount of islet tissue.

Blood-sugar estimations. Hoffmann & Anselmino [1933] found that the injection of preparations of their pancreotropic substance led to a slight fall of blood-sugar level during a few hours following injection. We have observed that the administration to rabbits of crude extracts of fresh ox anterior lobe often leads to a fall of blood sugar of about 10 mg./100 c.c. during the following 2 hr. Repeated daily injections of the same extract may, however, eventually lead to hyperglycæmia and glycosuria. In one instance out of more than a hundred, the blood sugar level of a rabbit fell to the convulsive level of 20 mg./100 c.c. as the results of four daily injections of crude extract; the injection of the same preparation of extract produced the symptoms of diabetes in a dog.

It seemed probable, from our results, that a crude saline extract of fresh ox anterior lobe, although diabetogenic in a dog, contains a substance which can stimulate islet-tissue hypertrophy in normal rats. The possibility that the hypertrophy of the islets of Langerhans was only a compensatory response to the diabetogenic action of the extract, prompter and more effective in the rat than in the dog, had therefore to be considered. For this reason the blood-sugar values of the rats used in these experiments were of interest.

Previous unpublished experiments [Young] had demonstrated the impossibility of obtaining, in rats, a consistent diabetogenic response to extracts which were potent when administered to dogs. In Table II are shown the results of blood-sugar determinations on groups of rats injected daily with 2 c.c. (equivalent to 1 g. of fresh gland) of a saline extract of fresh ox anterior lobe, for different periods. The animals were killed on the day after the last injection. The results lie close to the

TABLE II

Days injected	No. of animals	Average blood sugar mg./100 c.c.
0	12	122
1	6	111
2	6	90
4	6	90
6	6	116
9	6	88
13	4	124
19	4	114
21	12	135

normal range of blood-sugar levels in rats killed at different times, and certainly show no striking rise or fall. They do not provide support for the idea that the islet hypertrophy is a compensatory response to the diabetogenic action of the extracts used, although this possibility cannot be entirely ruled out at present.

DISCUSSION

An increase in islet tissue may be due to proliferation of existing islets, sometimes involving the fusion of small adjacent islets (see Pl. I, fig. 3), or to the formation of new islets. Any attempt to measure such an increase must take account of the mass as well as the number of the islets, since they vary so much in size. Estimates based solely on impressions gained from the microscopical inspection of serial sections of the pancreas, and stressing particularly the presence or absence of giant islets, are clearly of little value. Although this point was emphasized by Bensley [1912], the majority of investigators who report increases in islet tissue still assess their results by such a method. Bensley [1912] introduced a more accurate method applicable to small animals, by staining intravitam with neutral red or janus green. This method, however, merely allows an islet count to be made, and thus reveals only an increase in the number of islets without taking account of growth in those already existing. Moreover, unless the zymogen in the acinar tissue is previously exhausted (e.g. by secretin) it is difficult to pick out by this method the very small islets which, as the present results show (see below), may contribute very substantially to an increase in total islet-tissue.

The presence of giant islets may constitute evidence of the proliferation of individual islets, although the possibility that small islets may run together to form larger ones cannot be ruled out (see Pl. I, fig. 3). It is quite clear from the results of the present investigation that the

presence of giant islets is not, *per se*, evidence of a statistically significant increase in the amount of islet-tissue (see Pl. I, figs. 3, 4, 6). In our control animals the islets vary from small groups of about a dozen cells (micro-islets) to masses of greatest length 0·5 mm. There are no giant islets. In those experimental animals in which an increase of total islet tissue is recorded there is often a rise in the number of micro- and medium-sized islets, but sometimes the increase is apparently due almost entirely to an excess of micro-islets. Giant islets may be present in experimental animals, whether or not there is a significant increase in the total amount of islet tissue, as determinable by our method.

There is one observation which ought to be mentioned, since it appears to throw some doubt on the significance of our findings, and to necessitate some hesitation in drawing conclusions from them. We have examined the pancreases of several uninjected Norwegian hooded rats, and found to our surprise that the islet/acinar ratios were equal to or above the highest figures in our experimental series. Our experiments, however, were made entirely on Wistar rats, used, as far as possible, in groups of ten, and the ten control animals showed remarkably constant ratios of islet to acinar tissue. Without speculating on the possible cause of the difference between the two breeds of rat, we must emphasize the fact that our results are only significant with regard to differences between control and experimental rats of the strain we used.

As the total amount of acinar tissue appears to be slightly increased in most of our experimental animals, it seems that the effect of the "alarm reaction" [Selye, 1937] plays no important part in these results. In any case, adaptation would have occurred after 3 weeks [cf. Selye, 1937].

We believe that those authors [e.g. Anselmino *et al.* 1933] who claim to have demonstrated a "pancreotropic" activity of pituitary extracts on the basis of the histological appearance of the islet tissue after the administration of such extracts, cannot be considered to have done so. The histological appearance of serial sections of the pancreas, and the presence of giant islets, can be extremely misleading, and we ourselves were completely deceived by the preliminary results of experiments with "tethelin". Giant islets were present in the pancreases of injected rats, and the general appearance of the islets was such as to suggest that proliferative activity had been stimulated (cf. Pl. I, fig. 6). Nevertheless, analysis of the results for the islet/acinar ratio, for a group of eleven animals, showed that the mean figure was not significantly greater than that for the control group.

Anselmino *et al.* [1933] prepared their "pancreotropic substance" from acetone-dried fresh anterior pituitary glands, but we have been unable to obtain a significant increase in islet-acinar ratio using acetone-desiccated material (cf. Text-fig. 1). On the other hand, we found that a saline extract of fresh, undesiccated gland was active, and as such an extract possesses strong "anti-insulin" activity we are unable to agree with Chrzanowski & Grzycki [1937] that the action of the "pancreotropic substance" is inhibited by the "contra-insulin" factor.

Koster [1930] states that the pancreas of a hypophysectomized dog is usually atrophic, but so far as we are aware there is no satisfactory evidence of a specific islet-tissue atrophy following hypophysectomy. Indeed, as already noted, Krischesky [1936], using a quantitative method of islet-tissue assay, found that hypophysectomy actually increased the amount of islet tissue in the pancreas of the rat. To speak of a pancreotropic hormone is therefore at present quite unjustified.

We do not know whether a saline extract of fresh anterior lobe contains a specific substance causing islet-tissue hypertrophy, or whether this activity is associated with one of the known pituitary hormones. We have failed to find evidence that the increase of islet tissue, in our experiments, is due to a compensatory response to the diabetogenic activity of crude extracts, but until the "pancreotropic substance" has been separated from other pituitary factors its individuality must remain in doubt.

There is no evidence that the slight hypoglycæmic action of crude pituitary extract, observed by us and other investigators, is of physiological significance and is due to stimulation of insulin secretion by the pancreas; until further evidence has been obtained it would seem wise to suspend judgement. It should be pointed out that there is as yet no evidence that any islet tissue formed under the influence of pituitary extracts is capable of secreting insulin.

Some comment may here be made on the question of the histogenesis of the islets of Langerhans. Several recent authors [Florentin & Picard, 1936*a*; Bierring, 1934, etc.] have revived the older concept of the transformation of exocrine acinar tissue into endocrine islet tissue, as a factor in hypertrophy of the islet tissue. The histological appearances, from which these authors deduce this transformation, may be described briefly as irregularity in the contours of the islets, so that there appears to be an intermingling of the acinar zymogenic cells and islet cells, and the appearance of isolated fragments of basophil acinar tissue in the interior of some of the larger islets (see Pl. I, figs. 4, 6). Occasionally a cir-

cumscribed area of acinar tissue appears to be losing its characteristic basophil cytoplasm and zymogen and, between the atypical acini, there is an obvious proliferation of smaller cells resembling true islet cells (see Pl. I, fig. 5). We have observed these phenomena so rarely in the present material that we are unable to accept the transformation of acinar tissue into islets, if it occurs at all, as a constant factor in proliferation. In the absence of more critical cytological preparations, from material in which these atypical appearances are very common and can be induced consistently by experimental means, we do not wish to express an opinion on this important issue. Nevertheless, we wish to point out that those who believe that, in some circumstance, islet tissue may arise by the transformation of acinar tissue, have done little to answer the criticisms of this theory advanced in Bensley's classical paper [1912] on this subject.

Summary

1. A quantitative method of evaluating the ratio islet tissue/acinar tissue for the pancreas of the rat has been applied to the question of the "pancreotropic" [Anselmino & Hoffmann] action of an extract of ox anterior pituitary gland.

2. The mean value of this ratio for a group of eleven Wistar-strain white rats injected daily for 2–3 weeks with a saline extract of fresh gland was double that for ten control animals: the difference between these mean values is shown to be statistically significant. As the total amount of acinar tissue was increased rather than decreased, the increase in the ratio islet tissue/acinar tissue must indicate an increase in the absolute amount of islet tissue.

3. The results of Anselmino *et al.* were not confirmed, in that a significant increase in the mean value of the islet/acinar ratio was not found for a group of ten rats receiving an aqueous extract of acetone-desiccated gland.

4. In numerous instances the pancreases of experimental animals exhibited giant islets and a histological picture which has been interpreted by many investigators as indicative of proliferative activity of islet tissue. Since, however, such an appearance was frequently not accompanied by an increase in the islet/acinar ratio, it cannot be cited as satisfactory evidence of a significant increase in the amount of islet tissue.

5. While the increase observed in the Wistar-strain rats we have used appears to be statistically significant, the fact that normal rats of the

only other strain examined showed a widely different islet/acinar ratio suggests the necessity of caution in drawing any general conclusion.

6. The extract which increased the islet/acinar ratio of the pancreas had little or no effect on the blood-sugar level of our rats. The significance of this result is discussed.

The photomicrographs are the work of Mr F. G. Pittock, F.R.P.S., to whom we express our thanks.

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DESCRIPTION OF PLATE I

Fig. 1. A typical section (maximum size) taken from the largest part (base) of a block. The pale staining islets stand out in strong contrast to the darker acinar tissue. D = duct, F = connective tissue, and fat, L = lymph glands, M = mesentery. × 2·9.

Fig. 2. A giant islet. Rat injected with fresh gland extract for 3 weeks; islet/acinar ratio = 3·2. Note the micro-islet at "X". × 32·5.

Fig. 3. Some giant islets appear to be formed from the fusion of smaller adjacent islets. Rat injected with fresh gland extract for 3 weeks; islet/acinar ratio = 1·5, i.e. within control limits. × 47.

Fig. 4. A giant islet showing apparent intermingling of islet and acinar cells at the periphery. Rat injected with dry-gland extract; islet/acinar ratio = 1·2. × 47.

Fig. 5. Showing a lobule of acinar tissue in which the acinar cytoplasm is less basophil, the zymogen is depleted and there is a proliferation of smaller cells resembling islet cells between the transformed acini. Rat injected with fresh gland extract for 3 weeks; islet/acinar ratio = 4·5. × 32·5.

Fig. 6. In this large islet there is an apparent isolation of small acini surrounded by islet tissue but in serial sections the continuity of these acini with the remainder outside the islet has been clearly traced. Rat injected with tethelin for 2 weeks; islet/acinar ratio = 1·8. × 47.

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EFFECTS OF CASTRATION AND OF SEXUAL HORMONES ON THE ADRENALS OF MALE RATS

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The occurrence of hypertrophied "castration adrenals" in male rats has been observed previously. The literature on the subject, which is scanty, is referred to by Andersen & Kennedy [1933], who found a wider cortex in "castration adrenals" with a less clearly demarcated zona glomerulosa and approximately the same distribution of lipoids as compared with normal controls. As to the precise effects of castration on lipoid content, the authors are indefinite. More recently increase in weight of "castration adrenals" in male rats has been observed by Winter & Emery [1936].

The effect of injections of the hormones mentioned below has been studied only on the X-zone (not present in most animals including the rat and human being) of castrated mice by Deanesly & Parkes [1937] and Cramer & Horning [1937]. Callow & Deanesly [1935] could not find changes in the adrenals of mice injected with androsterone. The X-zone is only temporarily present in the female mouse, disappearing rapidly after the first pregnancy or with age. The zone does not exist in the adult male mouse, but is produced by castration [Deanesly, 1928; Cramer & Horning, 1937]. Deanesly & Parkes [1937] and Cramer & Horning [1937] found that the appearance of this zone in castrated males can be inhibited, or, if already present, caused to disappear by injections of androstenediol, testosterone, testosterone propionate and androstanediol.

In previous papers [Korenchevsky, 1930, 1932; Korenchevsky & Dennison, 1934] the effects were studied of cryptorchidism and castration on the weights of different organs including the adrenals. Castration before sexual maturity resulted in hypertrophy of the adrenals. This was confirmed in our succeeding papers, in which it was also shown that the

"castration hypertrophy" of adrenals decreases or disappears after injections of hormonal extracts from the male urine [Korenchevsky *et al.* 1933] and of the following pure sexual compounds: androsterone and androstanediol and their water-soluble esters [Korenchevsky, 1935; Korenchevsky *et al.* 1935], transdehydroandrosterone [Korenchevsky & Dennison, 1936], testosterone [Korenchevsky, 1936; Korenchevsky *et al.* 1936], testosterone propionate [Korenchevsky & Dennison, 1937; Korenchevsky *et al.* 1937*b*], Δ^4-androstenedione and Δ^5-androstenediol [Korenchevsky *et al.* 1937*a*]. Testosterone propionate, injected into normal male rats, did not produce definite changes in the size or weights of the adrenals [Korenchevsky *et al.* 1937*c*].

In the present paper the results are given of histological examination of the adrenals of rats described in the above-mentioned papers of Korenchevsky and co-workers. In the same papers the details of the experiments can be found. Our histological investigation was made on the adrenals of 53 normal and 150 castrated rats. The age of the rats, number of animals in each group, definition of the group, hormones injected, average weight of adrenals and width of the cortical zones and of the medulla are given in Tables I and II.

A short account of the results obtained has been given elsewhere [Hall & Korenchevsky, 1937].

Technique

The rats were castrated when 21–27 days old, usually 21–24 days, i.e. before sexual maturity. All the sexual compounds used were artificially prepared and supplied by Messrs Ciba, Ltd. They were dissolved in sesame oil and the daily dose was injected subcutaneously twice a day in half doses. The period of the injections was 21–23 days for most of the hormones, but in about half the experiments with androsterone and androstanediol it was longer—27–59 days. At the end of the experiments the rats were anæsthetized with ether and then killed by bleeding from the abdominal aorta.

Special care was taken to dissect the adrenals and drop them into Bouin's fixative as soon as possible after death in order to prevent destructive changes in the cells. The paraffin sections were stained with hæmatoxylin and eosin, and frozen sections for lipoids with Scharlach Red.

Micrometer measurements were made at the widest diameter of the adrenals, both opposite sides of each of the cortical zones were measured, and the mean of these measurements was taken. The figures are given in Tables I and II. In Table II the weights of the adrenals of rats receiving different doses giving similar effects were averaged and therefore these data differ a little from the detailed weights given for each dose of the hormones in our previous papers, from which these data were taken. The adrenals of rats having pneumonia or other diseases were not investigated since these diseases usually change their structure. The drawings were made by Miss K. Hall from areas in the peripheral one-third of the zona fasciculata and the middle part of the zona reticularis, and were done with the aid of a camera lucida. The photomicrographs were made by Mr W. Pereira.

Adrenals of normal uninjected male rats

In the rat's adrenal cortex, three zones are usually differentiated: zona glomerulosa, fasciculata and reticularis. We have invariably found a fourth zone, the "demarcation zone". This zone has been already observed by Bonnamour [1905*a*] and Jackson [1919, "transition band"] in the rat, and by Babes & Jonesco [1908] in the dog. Mulon [1912], however, in the dog found large lipoid granules in this region of the adrenal. It is situated between the zona glomerulosa and fasciculata and consists of 1–4 rows of cells, considerably smaller than those of the fasciculata and usually slightly smaller than those of the glomerulosa (Fig. 1). In sections embedded in paraffin this zone is conspicuous, because in most of the cells vacuoles are absent or only few and small. The greatly vacuolated cells occasionally seen in this zone are most probably explained by penetration of cells from the fasciculata or glomerulosa. In frozen sections stained with Scharlach Red the demarcation zone is still more conspicuous, its cells almost entirely free from lipoid granules, while the cells of the adjoining zones, glomerulosa and fasciculata, are crowded with granules (Fig. 7).

Effect of castration

The data are given in Table I and Figs. 3 and 8. The changes appear gradually and are fully developed about 2 months after castration, in 80-day-old rats.

The increase in weight and size of the "castration adrenal" is due to hypertrophy of the zona fasciculata and reticularis, especially the latter, resulting from a considerable increase in the size of the cells composing these layers. The greater hypertrophy of the reticularis may possibly be explained by hyperæmia and also perhaps by hyperplasia.

In paraffin sections vacuolation is shown to increase slightly after castration (cp. Figs. 2 and 3) and is of a more even character as regards the numbers and size of the vacuoles, especially in the zona fasciculata. Staining with Scharlach Red shows a definite change in the demarcation zone which becomes impregnated with lipoid granules and therefore appears to be absent, its cells being indistinguishable from those of the adjoining zones. A similar impregnation of this zone with lipoids was described by Jackson [1919, p. 264] in rats after inanition.

The medulla of castration adrenals was on the average only slightly larger. Since, however, the individual variations in width of medulla

were considerable, it is difficult to draw a definite conclusion without more numerous measurements. With the stains used, no changes were noticed in the cells of the medulla.

Effects of injections of sexual hormones into castrated rats

The data given in Table II (injected rats) have to be compared with those in Table I (control normal and castrated rats). The effect produced by all the hormones investigated was similar, varying in degree or in

Table I. Effect of castration on weight of adrenals and width of cortical zones and of medulla. N. = normal, C. = castrated rat

No. of group and age in days	No. of rats		Average weight of adrenals mg.		Average width, μ							
					Glomerulosa		Fasciculata		Reticularis		Medulla	
	N.	C.	N.	C.	N.	C.	N.	C.	N.	C.	N.	C.
I 40–49	9	2	41	55	67	69	585	718	301	284	1303	1503
II 60–69	5	6	48	77	67	59	635	685	301	418	1336	1420
III 70–79	4	11	54	77	67	69	568	651	384	434	1319	1436
IV 80–89	10	12	58	79	67	69	568	651	384	451	1319	1486
V 90–99	7	9	65	79	67	69	586	668	334	451	1386	1486
VI 100–149	6	4	54	69	67	69	568	634	351	451	1436	1420
Average	—	—	53	73	67	67	585	668	342	415	1350	1459

Table II. Effect of injection of sexual hormones on weight of adrenals and width of cortical zones and of medulla

No. of group and age in days	Hormone injected	Daily doses used mg.	No. of rats	Average weight of adrenals mg.	Average width, μ			
					Glomerulosa	Fasciculata	Reticularis	Medulla
I 77–149	Androsterone	0·45–0·9	7	56	75	618	317	1403
II 64–88	Androsterone	1·8–3·6	12	52	69	534	284	1286
III 91	W.S. androsterone	13·6–27	2	44	75	534	267	1386
IV 49–83	Androstanediol	0·175–0·35	9	49	75	551	317	1253
V 64–88	Androstanediol	0·7	5	49	69	584	284	1219
VI 49–91	W.S. androstanediol	1·5–5·3	6	51	69	601	267	1219
VII 68–86	Transdehydroandrosterone	1·4	19	64	59	618	351	1420
VIII 79	Androstenedione	0·5–3	11	55	69	601	367	1336
IX 69	Androstenediol	0·2–0·6	4	53	69	618	384	1319
X 98	Testosterone	0·033–0·167	8	72	69	668	351	1436
XI 98	Testosterone	0·5–1·41	8	63	75	585	367	1402
XII 98	Testosterone + androsterone	0·167–0·5 / 0·45	4	68	75	668	351	1470
XIII *89	Testosterone propionate	0·167–1·4	6	56	69	620	351	1403
XIV *89	Testosterone propionate	0·167–1·4	5	54	75	585	367	1403
XV 102	Testosterone propionate (normal rats)	0·5–1·5	12	61	59	620	434	1386

* The rats in group XIII were killed 1 day, and in group XIV 9 days after the last injection of testosterone propionate.

details only. It is noteworthy that even dehydroandrosterone, which has the weakest stimulating effect on the sexual organs, has a pronounced effect on the adrenals.

Medulla

With the stains used, no definite changes were found in the cells of the medulla and therefore the following description is of the cortical changes only. On the average, however, the width of the medulla (Tables I and II) in most of the injected groups was slightly smaller than in the control castrated rats, returning to the normal average figure.

Cortex

Width of cortical zones. All the hormones used, if injected in sufficiently large doses, caused the hypertrophied fasciculata and reticularis to return to or towards normal (Tables I and II). Complete recovery to normal width was obtained with androsterone, androstanediol and testosterone. Transdehydroandrosterone, androstenedione and testosterone propionate caused the zona reticularis to return completely to normal, but the fasciculata only partially; while androstenediol, in the doses used, in most rats produced only a partial recovery towards normal width in both zones.

It is important, however, to emphasize, that after injections of some of the hormones, the zones became abnormally narrow, e.g. the fasciculata with large doses of androsterone, and the reticularis with all doses of androsterone and androstanediol.

Size of the cells. The cells were smaller after the injections, becoming normal in size or less than normal (Fig. 6), there being in general correspondence between the changes in width of the zones and size of the cells. It is to be emphasized, however, that, with the exception of transdehydroandrosterone and the smallest dose of testosterone (0·033 mg., Fig. 5), all other hormones, in the doses used, reduced the size of the cells to normal, or smaller (Fig. 4), but in some rats there was only a partial recovery in the width of the zones. This partial recovery suggests that, besides hypertrophy of the cells, other factors may play a part in hypertrophy of the cortex, e.g. hyperæmia of the adrenals or hyperplasia of the cortical cells.

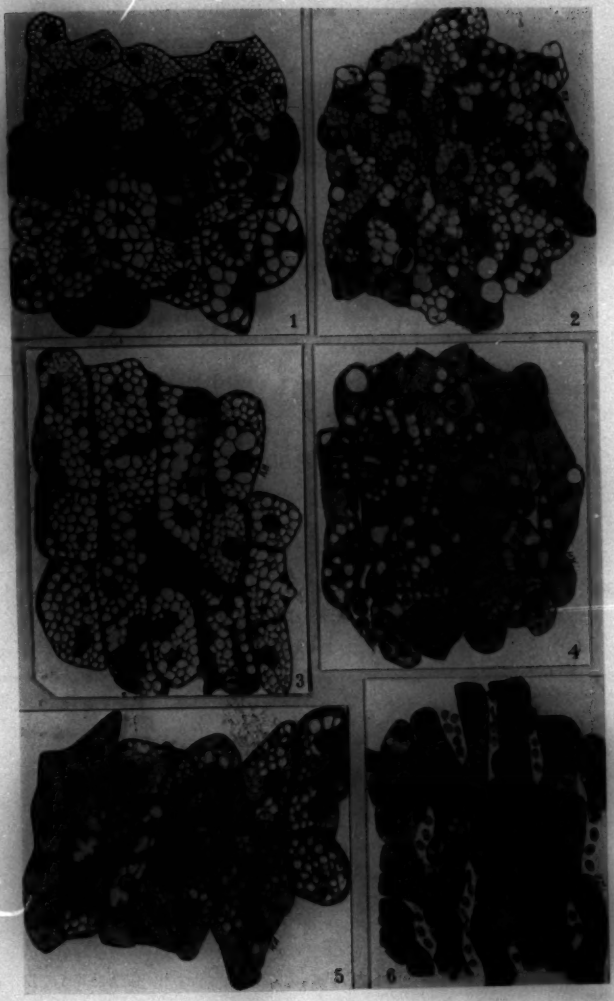
Vacuolation (as seen in paraffin preparations). After the injections, the vacuoles (with the exceptions mentioned below) decrease in number, becoming sometimes normal but more often fewer (Figs. 4 and 5), or even considerably fewer than normal (Fig. 6). Usually the size of the vacuoles also decreases. After daily injections of 0·9 mg. or more of androsterone (Fig. 6), the fasciculata cells become almost unrecognizable

owing to their greatly reduced size and the almost complete disappearance of vacuoles. With most hormones, the higher the dose, the less marked becomes the vacuolation. Exceptions are testosterone and to a lesser degree testosterone propionate, smaller doses of which decrease vacuolation more than do larger doses.

Lipoid granules (preparations stained with Scharlach Red). All the hormones in all doses used caused a decrease below normal in the number of lipoid granules in the reticularis and especially in the fasciculata (Figs. 9–11). The greater the dose, the more pronounced was the reduction. In some cases (e.g. 0·033 and especially 0·167 mg. of testosterone) small "patches" of the fasciculata remained much impregnated with lipoid granules, which had disappeared, more or less evenly, from the larger neighbouring areas (Fig. 9). The lipoid granules in the zona glomerulosa, however, are much less decreased by the injections and remain proportionately more numerous in this zone than in the fasciculata and reticularis. In fact in some cases (e.g. 0·9 mg. androsterone, Fig. 10) the number and size of the lipoid granules in the zona glomerulosa remain apparently unchanged, being in conspicuous contrast to the lipoid-impoverished fasciculata and reticularis. Jackson [1919, p. 250] describes a similar change in the adrenals of starving rats, while our injected rats had good appetites, and their gain in weight was greater than in the controls [Korenchevsky *et al.* 1935, p. 2540]. Such a difference in the resistance to the action of hormones suggests a difference in the chemical nature of the lipoids in different zones and, perhaps, a difference in their endocrinological function.

Demarcation zone. All doses of all hormones injected caused this zone in castrated rats to become conspicuous again, since the lipoid granules were absorbed and the vacuoles disappeared (Figs. 9–11). Thus the zone returns to normal.

Discrepancy between vacuolation and lipoid granules content. As a general rule, when vacuolation in the cortical cells is decreased, the number of lipoid granules as shown by lipoid staining is also decreased, and vice versa. This is, however, not always the case, since the lipoids might be present in different chemical forms, some showing positive and some negative staining reaction [Bonnamour 1905*b*; Grollman, 1936]. In our experiments an example of a discrepancy between vacuolation (pronounced) and number of lipoid granules (few) was observed after the injection of 1·4 mg. of testosterone and (to a lesser degree) of testosterone propionate. We have not made a detailed study of the nature of this discrepancy.



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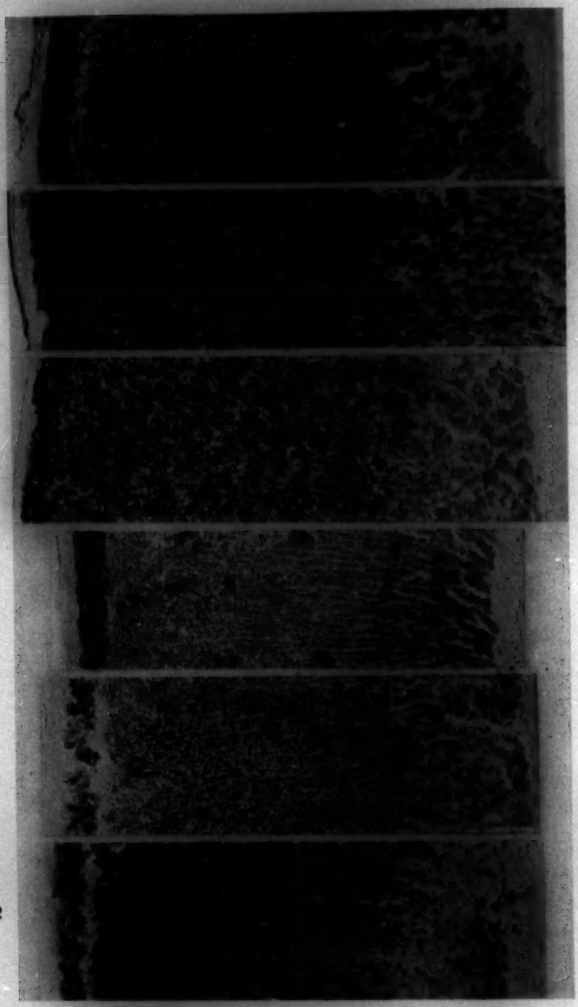
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The effect of a resting period of 9 days after testosterone propionate injections into castrated rats

When the adrenals of rats killed the day after the last injection (group XIII, Table II) were compared with those of their litter-mates killed 9 days after the last injection (group XIV), it was found that in both groups the weight and size of the adrenals returned to normal, the width of the zones (Figs. 11–12) and the size of the cells were nearly or completely normal, but there were the following striking differences. The adrenals of group XIII showed the abnormal effects usually produced by the injections, namely, decreased vacuolation and a very striking decrease, considerably below normal, in the number of lipoid granules (Fig. 11). In group XIV (Fig. 12) these abnormal features had completely or, mostly with large doses (0·5 mg.), almost completely disappeared 9 days after the last injection, and the adrenal appeared to be normal. These results show that the abnormal effects of the injections of sexual hormones disappear only some time after the cessation of the injections. At the same time, however, the experiments show that testosterone propionate may have a complete recovery effect on the adrenals of castrated rats, and also that this effect may be a comparatively lasting one, being present probably more than 9 days after the last injection was given.

The effects of testosterone propionate on the adrenals of normal rats

The data are given in group XV, Table II.

Although in injected rats the changes varied in degree, in most cases they were less pronounced with 0·5 than with 1·5 mg. The width of the zona fasciculata and reticularis, and the size of the individual cells were normal or slightly increased, a different reaction from that produced by the same hormone on the "castration adrenal". There was a definite decrease in normal vacuolation and in the numbers of lipoid granules (often strikingly reduced) as compared with those in the control uninjected litter-mates. In some cases the lipoid depletion took the same form as with testosterone in castrated rats, resulting in an uneven "patchy" appearance. In the zona fasciculata there was an increased number of degenerated nuclei with poorly developed and faintly stained chromatin networks.

SUMMARY

1. A histological investigation was made of the adrenals of 53 normal and 150 castrated rats, uninjected or injected with androsterone, androstanediol, transdehydroandrosterone, testosterone, testosterone propionate, Δ^5-androstenediol, and Δ^4-androstenedione.

2. Besides the zona glomerulosa, fasciculata and reticularis, a fourth zone, the demarcation zone, is constantly present in the cortex of the rat adrenal.

3. The following histological changes in the hypertrophied "castration adrenal" were observed: increase in the width of the zona fasciculata and reticularis, considerable increase in size of the individual cells, and a slight increase in vacuolation: an apparent disappearance of the demarcation zone due to the vacuolation of its cells and their impregnation with lipoid granules.

4. Injection into castrated rats of all the sexual hormones investigated produced a comparatively lasting recovery (at least 9 days) towards, or to, normal; or, usually with larger doses, the following abnormal changes in the adrenal cortex: a decrease below normal in the width of the zona fasciculata and the reticularis and of the size of the individual cells; abnormal decrease of vacuolation and of the number of lipoid granules.

5. The abnormal effects suggest some toxicity for adrenals of large doses of the hormones, but these effects had disappeared in most cases 9 days after the last injection and the adrenals had returned to normal.

6. The fact that the lipoids of the zona glomerulosa and those of the other three zones react differently suggests that they are not similar and may perhaps have a different endocrinological significance.

7. The chief effects of the doses used of testosterone propionate on the adrenals of normal rats were reduction of vacuolation and number of lipoid granules in the cells of the zona fasciculata and reticularis.

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DESCRIPTION OF PLATES I AND II

Plate I

Fig. 1. Normal uninjected control rat. Upper rows of vacuolated cells belong to zona glomerulosa; medium rows of small unvacuolated or partly vacuolated cells—demarcation zone; lower rows of large vacuolated cells—zona fasciculata. Rat 382 g. 99 days old. Adrenals 61 mg. ×545. Hæmatoxylin and eosin.

Fig. 2. Normal uninjected control rat. Cells of zona fasciculata, with uneven vacuolation, and occasional degeneration of nuclei. Rat 365 g. 99 days old. Adrenals 52 mg. ×545. Hæmatoxylin and eosin.

Fig. 3. Castrated uninjected control rat. Cells of zona fasciculata much enlarged, with greater but more even vacuolation than in Fig. 2. Rat 325 g. 99 days old. Adrenals 82 mg. ×545. Hæmatoxylin and eosin.

Fig. 4. Testosterone propionate (0·167 mg. daily × 23 days) injected castrated rat. Zona fasciculata. Medium effect. Cells are smaller or equal to normal without any or with reduced vacuolation. Rat 308 g. 84 days old. Adrenals 61 mg. ×545. Hæmatoxylin and eosin.

Fig. 5. Testosterone (0·033 mg. daily × 23 days) injected castrated rat. Zona fasciculata. Comparatively weak effect of small dose. Cells smaller than in Fig. 3. Vacuolation reduced below that in castrated (Fig. 3) and normal (Fig. 2) rats. Rat 338 g. 93 days old. Adrenals 73 mg. ×545. Hæmatoxylin and eosin.

Fig. 6. Androsterone (0·9 mg. daily × 47 days) injected castrated rat. Zona fasciculata. Strong effect. Cell-cords narrower, making blood vessels more conspicuous. Cells reduced in size much below normal. Vacuoles few or absent. Rat 258 g. 77 days old. Adrenals 39 mg. ×545. Hæmatoxylin and eosin.

Plate II

Fig. 7. Normal control uninjected rat. From left to right: (1) unstained adrenal capsule; (2) zona glomerulosa—rich in lipoid granules (black); (3) demarcation zone, very narrow—lipoid granules few or absent; (4) zona fasciculata—widest zone, with columnar arrangement of cells rich in lipoids; (5) zona reticularis—narrow network of cells, with fewer lipoid granules than in zona fasciculata and glomerulosa; (6) unstained medulla. Rat 323 g. 99 days old. Adrenals 59 mg. ×72. Scharlach Red.

Fig. 8. Castrated control uninjected rat. Demarcation zone appears absent because of impregnation with lipoids. Because of the hypertrophy only the upper part of zona reticularis is seen. Rat 325 g. 99 days old. Adrenals 82 mg. ×72. Scharlach Red.

Fig. 9. Testosterone (0·033 mg. daily × 23 days) injected castrated rat. Weak effect of small dose. Glomerulosa with normal amount of lipoids, fasciculata impoverished in "patches", less lipoid in reticularis. Reappearance of demarcation zone. Rat 338 g. 93 days old. Adrenals 73 mg. ×72. Scharlach Red.

Fig. 10. Androsterone (0·9 mg. daily × 47 days) injected castrated rat. Strong effect. Lipoid content in glomerulosa normal, but much decreased in fasciculata and less in reticularis. Rat 258 g. 77 days old. Adrenals 39 mg. ×72. Scharlach Red.

Fig. 11. Testosterone propionate (0·167 mg. daily × 23 days) injected castrated rat, killed 1 day after last injection. Strong effect. Number of lipoid granules much decreased in all zones. Effect much stronger than that with the same dose of testosterone. Rat 327 g. 73 days old. Adrenals 58 mg. ×72. Scharlach Red.

Fig. 12. Testosterone propionate (0·167 mg. × 23 days) injected castrated rat, killed 9 days after last injection. Number of lipoid granules restored to normal in fasciculata, slightly below normal in reticularis and glomerulosa. Rat 267 g. 73 days old. Adrenals 54 mg. ×72. Scharlach Red.

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THE RESPONSE OF A DENERVATED GANGLION TO ACETYLCHOLINE

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In a recent paper [Brücke, 1937] I have shown that preganglionic denervation of the superior cervical ganglion of the cat causes the disappearance of a large proportion of its cholinesterase. Brown & Feldberg [1936] had shown that, under the same conditions, the ganglion loses most of its normally large load of acetylcholine, as Engelhart [1931] had earlier found that degeneration of the postganglionic ciliary nerves, following removal of the ganglion, caused the disappearance of acetylcholine from the iris and ciliary body of the cat's eye. Postganglionic ciliary denervation sensitizes the iris to the constrictor effect of acetylcholine [Shen & Cannon, 1936], and the sensitization of the iris to adrenaline by sympathetic denervation has been a familiar phenomenon since Meltzer & Auer [1904] first described it. More nearly analogous, however, to the denervation of the ganglion by degeneration of its preganglionic fibres, is the denervation of a voluntary muscle by degenerative section of its motor nerve, which has long been known to produce a great sensitization of the muscle fibres to acetylcholine [Frank *et al.* 1922; Dale & Gasser, 1926]. There was no lack of analogy, in any case, to suggest the enquiry, whether preganglionic denervation, with the attendant loss of cholinesterase, would sensitize the cells of the ganglion to the stimulating action of acetylcholine, injected or applied artificially.

This question has already been the subject of experiments by Cannon & Rosenblueth [1936], who obtained results which they regarded as demonstrating such sensitization. They cut one cervical sympathetic

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nerve of a cat and allowed time for degeneration. For the final experiment, they tied the suprarenal veins, gave atropine to remove the muscarine action of acetylcholine and curare to prevent contraction of the extrinsic eye muscles, and recorded the contractions of the nictitating membranes. In other experiments they applied acetylcholine directly to the surfaces of the exposed ganglia, without giving atropine or curare. When acetylcholine was thus injected or applied, the nictitating membrane on the side of preganglionic denervation gave a well-marked contraction, though the doses and concentrations were so chosen as to cause no distinct contraction of the membrane on the normal side. This could not by itself, however, be taken as proving a sensitization of the ganglion cells to acetylcholine, since even preganglionic denervation causes an enhanced peripheral reaction of the pupil and the nictitating membrane to adrenaline—a phenomenon originally observed by Meltzer & Auer [1904] and more systematically studied by Hampel [1934]; and the earlier work of Cannon and his colleagues justifies the assumption that preganglionic denervation will similarly sensitize the peripheral tissues to the natural sympathetic transmitter, "sympathin". Cannon & Rosenblueth, however, made tests of this enhancement of the reaction to adrenaline, in comparison with that to acetylcholine. In the experiments illustrated in their paper, the dose of adrenaline injected caused a just perceptible response of the membrane on the normal side, and a contraction of that on the side of denervation which, though greatly enhanced, was of rather less amplitude than that which the same membrane gave in response to an injection of acetylcholine causing, as already mentioned, no visible response on the normal side. Such a comparison, by itself, might suggest some sensitization of the reaction of the ganglion cells to acetylcholine, in addition to the peripheral sensitization to adrenaline or sympathin, as the result of the preganglionic denervation; and Cannon & Rosenblueth have given it this interpretation. It seemed doubtful, however, whether such a comparison of the effects of doses which, in both cases, were near to the threshold of response on the normal side, was sufficient to justify such a conclusion. Accepting the assumption that the sensitization of the peripheral structures to adrenaline would be identical with that to sympathin, if both were similarly administered, we cannot, without further evidence, assume that the same proportionate increase will be observed in the response to adrenaline reaching the plain muscle cells by slow diffusion from the blood, and in that to sympathin liberated in close relation to them at the endings of the postganglionic nerve fibres. The time relations of the two effects are

obviously different, and an enhancement of the relatively rapid and evanescent response, to nerve impulses evoked from the ganglion cells by acetylcholine, might well show itself chiefly by an increased amplitude of contraction, whereas the factor of duration might figure strongly in the enhancement of the slower response to adrenaline. To justify a conclusion, that the response to the transmitter of nervous effects, given by effector cells sensitized to its action, is inadequate to account for the observed difference, and that the ganglion cells must, in addition, be abnormally sensitive to acetylcholine, we should, at least, require evidence that the enhancement of the response to acetylcholine is constantly and conspicuously greater than that of the response to adrenaline, and that the superiority is evident also with doses of both drugs which are not near to, or below, the threshold for the normal side.

I have endeavoured, accordingly, in my experiments, to cover a wider range of doses, both with acetylcholine and with adrenaline. If the dose of acetylcholine, injected or applied, is large enough to cause a measurable reaction also of the normal nictitating membrane, then the difficulty of comparison should be reduced; since, although the response to acetylcholine will have different time relations from that to adrenaline, the difference will be present on both the normal and the denervated sides, and the reactions of the normal membrane, under such conditions, afford a basis for estimating increases, of peripheral excitability only in the case of adrenaline, and of peripheral *plus* ganglionic excitability in the case of acetylcholine. If, therefore, ganglionic sensitization does occur after degeneration of the preganglionic sympathetic fibres, a dose of acetylcholine, which on the normal side produces a contraction of the nictitating membrane approximately equivalent to that produced by a given dose of adrenaline, should, on the denervated side, produce an obviously greater response than that evoked there by the same dose of adrenaline.

In making such comparisons, and interpreting their results, it has further to be borne in mind that the proportion, in which the reaction on the denervated side is enhanced, is not constant for all doses of adrenaline, but tends to diminish above a certain level of dosage, and may even disappear with doses of sufficient magnitude. In the case of acetylcholine this phenomenon is accentuated by a secondary, depressant effect of large doses, to be later discussed. Most of my experiments were, accordingly, made with doses in a middle range, where the ratios are fairly constant.

Methods

In adult cats, one cervical sympathetic nerve was cut, under ether anæsthesia and with aseptic precautions, usually about a fortnight before the main experiment. A few centimetres of the nerve were removed as a precaution, and the completeness of the section was verified at the final experiment by stimulation of the peripheral cut end. For the main experiment the cats were anæsthetized with Dial (0·75 c.c. per kg. intraperitoneally) or by 0·05–0·06 c.c. per kg. of 1 p.c. solution of chloralose, given intravenously under preliminary ether, and followed by 0·5 g. per kg. of urethane intraperitoneally. When anæsthesia was complete, both suprarenals were removed through dorsal incisions. To permit unhampered recording of the movements of the nictitating membranes, the upper eyelids were incised between ligatures securing the tarsal arteries, the two inner halves being then drawn out of the way by tying their ligatures together across the root of the nose. The eyeballs were either left in situ, or removed, after division of the conjunctiva and tying and cutting the extrinsic ocular muscles and the optic nerve.

In order to record the movements of the nictitating membranes, fine steel wires or silk threads were attached to the free borders. The records were either of an approximately isometric type, made with a torsion-wire lever of the kind used by Sherrington & Eccles, or isotonic. In the isotonic records two similar levers were used, each carrying a load of 5 g. and giving a six to sevenfold amplification with frontal writing-points. The nictitating membranes were attached to them by silk threads running over pulleys, the cat being fixed in the usual supine position. For the isometric records the head and forequarters of the cat were turned into the prone position, the head being held rigidly in a clamp, with the orbits directly under the lever. The torsion-wire was fixed in the middle of a lever with two short, equal arms. To either end of this a nictitating membrane could be attached by means of a vertical steel wire, and a light aluminium extension transmitted to the recording drum the magnified movement of the lever, the writing-point moving either upwards or downwards with increasing tension, according to the arm of the lever with which connexion was made. The magnification was such, that a change of 5 g. in tension corresponded to an arc of 1·2 cm. on the record. The tension changes for the two membranes could thus be recorded independently, or both membranes could be attached simultaneously to opposite ends of the lever, so that any deviation represented a difference between the tensions on the two sides.

Injections of drugs were made either into the femoral vein, or, more frequently in the case of acetylcholine, into the innominate artery. For this purpose, a small glass capillary cannula, of the type used by Dale & Richards [1927], filled with saline and closed at one end by a short length of narrow rubber tubing, was pushed centrally into the right subclavian artery, the side branches of which had all been tied off. The inner end of this cannula rested just below the division of the innominate artery into the two common carotids. In cats having an abnormal distribution of the branches of the innominate artery, such that the left carotid artery arose more centrally from the common trunk than both the right carotid and the right subclavian artery, this method of arterial injections had to be abandoned. In most cases, however, it proved very satisfactory, since much smaller amounts of acetylcholine (e.g. 0·1 mg. in 0·2–0·3 c.c. of saline) than those required with intravenous injection were sufficient, even in the absence of eserine, to stimulate the ganglion cells in the superior cervical ganglion. With intravenous injection, and using the somewhat heavy isometric recording system, up to 5 mg. of acetylcholine had to be injected, in the absence of eserine, to get good records; with the isotonic levers the amount required was smaller, i.e. 1–2 mg. After sensitization with eserine, much smaller doses could be used, and 0·1–0·2 mg. then usually gave good reactions, even with intravenous injection. To prevent clotting round the opening of the arterial cannula, the blood of the cat was rendered incoagulable by an intravenous injection of 1 c.c. per kg. of an 8 p.c. solution of chlorazol fast pink.

In experiments in which acetylcholine was applied to the surface of the superior cervical ganglion, the two ganglia were exposed with as little interference with their blood supply as possible, a little piece of rubber sheet being slipped under each to localize the effect of the applied acetylcholine. A tracheal cannula was inserted, the larynx and underlying part of the œsophagus and pharynx were extirpated and closed by ligature, and the wound was held widely open by steel hooks. This method provides a very efficient exposure of the ganglia, and acetylcholine, in the chosen concentration, can be carefully painted on the whole extent of their upper surfaces.

The arterial blood pressure was recorded from a femoral artery. 1–2 mg. of atropine sulphate was given intravenously at the beginning of every experiment, to eliminate the depressant effects of acetylcholine on the circulation, and its other parasympathetic effects, including any which it might produce on the plain muscle moving the nictitating membrane. If, during the course of an experiment, the atropine effect

became too weak to prevent depressant effects of the acetylcholine injections, a further injection was given. Solutions of acetylcholine chloride and of eserine sulphate were made freshly for each experiment.

Experimental

(1) *Sensitization to adrenaline by preganglionic degeneration*

The great majority of our experiments showed a very definite, though individually rather varying augmentation of the reaction to adrenaline of the nictitating membrane, on the side on which degeneration of the cervical sympathetic nerve had been produced. In some exceptional cases this peripheral sensitization has, however, been found to be entirely absent, and in such cases injections of doses of adrenaline, varying from the smallest (2·5γ) to the highest, may evoke even smaller contractions of the preganglionically denervated membrane than of the normal one. These anomalous cases, in which preganglionic degeneration causes no sensitization of the reaction of the nictitating membrane to adrenaline, were apparently not encountered by Hampel [1934], in his systematic study of this sensitization in comparison with that following postganglionic degeneration. Although no explanation for the occurrence of such cases can be offered at present, they seemed to be of particular interest, since they offered an opportunity to observe whether conditions, in which the augmentation of the reaction to adrenaline was absent, would leave any augmented reaction to acetylcholine, which would clearly be due to ganglionic sensitization.

One condition, by which the excess reaction to adrenaline of the nictitating membrane on the side of preganglionic nerve-section could be regularly diminished, in the course of an experiment, was found in the removal of the eyeballs. This procedure was tried originally to eliminate interference, due to movements of the eyeballs by their voluntary muscles, from the records of the nictitating membranes, without using curare, which causes a definite depression of the response of the ganglion cells to acetylcholine [cf. Brown & Feldberg, [1935]]. The observed loss of the enhanced response to adrenaline was then made the subject of deliberate experiments, in which the responses of the two membranes to adrenaline were compared, before and after removal of the eyeballs. Fig. 1 reproduces a record from such an experiment, in which the upper line traces responses of the membrane of the normal side, the lower line that of the membrane on the side of preganglionic degeneration, the records being made with isotonic levers. At 1 the peripheral ends of the

cervical sympathetic nerves were stimulated, evoking a normal response on the normal side, and none on that of degeneration. At 2 are seen the responses of the membranes with the eyeballs still intact, to an intravenous injection of 30γ of adrenaline. The response on the side of denervation is clearly potentiated, though less conspicuously than with the same dose in many other cases. Between 2 and 3 the eyeballs were removed, and a second period of nerve stimulation at 3, similar to that at 1, shows that the removal has not affected the response of the normal membrane to such stimulation. At 4 are seen the responses now given by the nictitating membranes to a further injection of 30γ of adrenaline;

Fig. 1. Responses of normal (upper) and preganglionically denervated (lower) nictitating membranes of cat to adrenaline. Cervical sympathetic cut 14 days. (1) and (3), stimulation of both cervical sympathetics; coil at 16 cm. (2 V.). (2) and (4), 30γ adrenaline, intravenous. Between (2) and (3) both eyeballs removed.

the augmented response on the side of denervation has disappeared, the two nictitating membranes now showing practically identical reactions. Comparison of 2 and 4 shows that removal of the eye has not diminished the response of the normal membrane to this dose of adrenaline; it is the excess reaction of the membrane on the side of preganglionic denervation which has been eliminated. It seems as if a separate mechanism, possibly connected with the protrusion of the eyeball, contributes to this excess reaction, or is, in fact, entirely responsible for it. It is easy to demonstrate on the dead cat that movement of the eyeball out from the orbit, even when produced passively by traction on the cornea and with the eyeball rendered flaccid by removal of its contents, causes retraction of the nictitating membrane. Since it was known from the early work of

Meltzer & Auer that degenerative section of postganglionic fibres causes a greater sensitization to adrenaline than that produced by degeneration of the preganglionic fibres, it was thought possible that the sensitizing effect of the section of the cervical sympathetic nerve might, in reality, be due to the presence in it of some unrecognized postganglionic fibres with cell stations in the stellate ganglion. This possibility was excluded, however, by an experiment in which degeneration was produced by section of all the rami of the stellate ganglion, except those forming the annulus. Stimulation of the cervical sympathetic nerve was then entirely without effect on the nictitating membrane, and the membrane on the side of the degeneration showed an augmented response to adrenaline, just as after section of the cervical sympathetic nerve.

Whatever may be the explanation of this striking effect of the removal of eyeballs on the peripheral effects of adrenaline, it is clear that, if the section of the cervical sympathetic nerve sensitizes the superior cervical ganglion to the stimulating effects of acetylcholine, an enhanced response to this drug should persist after removing the eyeball. For, even if the greater peripheral response to adrenaline should be entirely confined to a mechanism involved in the protrusion of the eyeball, it is hardly conceivable that section of the preganglionic fibres should only affect those ganglion cells which send postganglionic fibres to that mechanism.

(2) *Effects of preganglionic denervation on the response to acetylcholine*

When the augmentation of the response to adrenaline was compared with that to acetylcholine, they showed a remarkable and practically invariable correspondence. Wherever, in a large number of experiments, a conspicuous augmentation of response to adrenaline was observed on the side of preganglionic denervations, a corresponding augmentation of the response to acetylcholine was seen. Fig. 2 is one from a number of examples of this kind. In the response at 1 to 30γ of adrenaline, and at 2 to 5 mg. of acetylcholine, injections being made intravenously in both cases, the lower tracings, from the side of preganglionic denervation, show conspicuously augmented responses; and it is not possible from measuring these responses to judge whether the enhancement was greater in the case of either drug than in that of the other. The relatively high dose of acetylcholine applied in this instance is made necessary by the absence of eserine or prostigmin, but the following example, taken from an experiment in which 1 mg. of eserine was given intravenously before

the injection of acetylcholine, shows that the same effects can be observed with amounts of acetylcholine of the order of those applied by Cannon & Rosenblueth after sensitization with prostigmin. In this case 0·3 mg. of acetylcholine given intravenously, and protected by eserine from the action of blood on its way to the ganglion, evoked a marked contraction on the denervated side, and a small but distinct and measurable one on the normal side. 5γ and 10γ of adrenaline gave reactions, which were, on both sides, smaller than the corresponding reactions to acetylcholine, but 30γ of adrenaline gave a reaction of the normal nictitating membrane which was exactly equal in tension to that which had been observed after

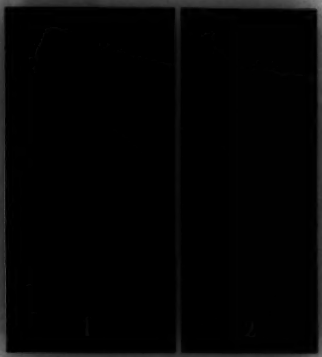

Fig. 2. Responses of normal (upper) and preganglionically denervated (13 days) nictitating membranes (lower). (1) 30γ adrenaline, and (2) 5 mg. ACh., both intravenous.

0·3 mg. of acetylcholine; and, here again, the response on the denervated side was just as great as, or even slightly greater than, that produced by the same dose of acetylcholine. 50γ of adrenaline were now injected and produced greater reactions on both sides, than those produced by 0·3 mg. of acetylcholine; but as the reaction on the normal side had increased relatively more than that on the denervated side, the difference, expressing the peripheral sensitization to adrenaline, was now smaller than with the dose of 30γ. This illustrates the necessity of a method of comparison which takes the reactions of the normal nictitating membrane as a basis for comparison.

Fig. 3 shows an example of the less frequent type of effect, in which preganglionic denervation, with the eyes still in position, has caused no increase, but even a diminution of the response to 30γ of adrenaline, as

seen at 2. In this case the reaction on the side of operation to 5 mg. of acetylcholine (in the absence of eserine), as seen at 3, is also less than that of the membrane on the normal side.

A similar correspondence of reactions is seen when the response to adrenaline and acetylcholine are tested before and after removal of the eyeballs. Fig. 4, which has been taken from the same experiment as

Fig. 3.

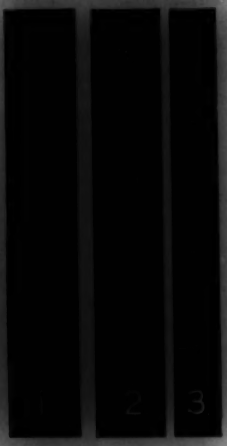

Fig. 4.

Fig. 3. As in Fig. 2, preganglionically denervated 16 days; (1) stimulation of cervical sympathetics ($c=10$); (2) 30γ adrenaline; (3) 5 mg. ACh., both intravenous. (No sensitization to either.)

Fig. 4. Effects of eserine and of removing eyeballs. Normal (upper) and preganglionic denervated (14 days) nictitating membrane (lower). (1) 4 mg. ACh.; between (1) and (2) 4 mg. eserine; (2) 2 mg. ACh.; between (2) and (3) both eyeballs removed; (3) 5 mg. ACh., all intravenous.

Fig. 2, illustrates these facts. At 1 the upper tracing records the response of the normal nictitating membrane to an intravenous injection of 4 mg. of acetylcholine, and the lower tracing that of the corresponding, denervated membrane, both eyes being in position. Between 1 and 2, 4 mg. of eserine were injected intravenously, and at 2, 10 min. after this injection, 2 mg. of acetylcholine gave a markedly potentiated response of both nictitating membranes. It will be seen, however, that this eserine potentiation is much more conspicuous on the normal side than on the

denervated side; it therefore tends to diminish the difference between the response of the normal and the denervated nictitating membrane. This phenomenon will be discussed later. About an hour after the injection of acetylcholine recorded at 2, when the effect of eserine had practically disappeared, the eyeballs were removed, and an intravenous injection of 5 mg. acetylcholine now gave the small contractions recorded at 3, which are nearly equal on the normal and the denervated sides, although, in this particular experiment, the removal of the eyeballs had produced only a great diminution, and not a complete abolition of the peripheral sensitization to adrenaline.

So far the evidence had produced no support for the suggestion that the ganglion cells were rendered sensitive to acetylcholine by degeneration of their preganglionic fibres. The increased response of the nictitating membrane appeared to be adequately explained by the peripheral sensitization to adrenaline, and therefore to the transmitter of sympathetic excitation. There remained, however, the possibility that the ganglion cells might exhibit, in addition, some direct increase of response to acetylcholine, which would only be detectable by a more quantitative system of recording. If the ganglion cells were also thus sensitized, it ought to be possible to demonstrate that the augmentation of the response of the nictitating membrane, to an injection of acetylcholine acting on the ganglion cells, was proportionally greater than the augmentation of its direct response to adrenaline. In the former case there would be an accentuation of response at two stages, in the latter at one only. For this purpose a number of comparative experiments were made with the isometric recording lever. Fig. 5 illustrates the result of an experiment of this kind. At 1, 2 and 3 the upper line represents the tension records from the membrane of the side of preganglionic denervation, the lower line those from the normal side. In each case the membranes were attached in succession to opposite ends of the torsion-lever, so that the record from the membrane of the operated side moves upwards, that from the normal side downwards with rise of tension, the initial tensions being practically equal in the two cases. In each case the records, though taken in succession, in response to equal injections, have been mounted in vertical correspondence, to save space and facilitate comparison. At 1 are recorded the reactions to initial injections of 5 mg. of acetylcholine intravenously, in the absence of eserine. Measurements show that the maximal increases of tension in the two cases are 2·73 g. (normal) and 5 g. (denervated), giving a ratio of 1 : 1·8. This is the accentuation which Cannon & Rosenblueth described, and it will be

seen that the reactions on both sides are of the type which they recorded, showing a quick rise to a maximum and a fall from it, initially almost as quick, but becoming slower when nearing the normal tension. At 2 the corresponding responses to intravenous injections of 70γ of adrenaline are recorded. The responses to adrenaline, reaching the plain muscle by slow diffusion from the blood vessels, have very different time relations from those to acetylcholine; the tensions after adrenaline take 5–6 min. to regain the normal. Again measuring the excess tension at its maximum in each case, we find 1·7 g. (normal) and 4·2 g. (denervated), with a ratio of 1 : 2·5, substantially greater than that between the responses to

Fig. 5. Isometric records. Normal (lower) and preganglionically denervated (17 days) nictitating membranes (upper). (1) 5 mg. ACh.; (2) 70γ adrenaline; (3) 6 mg. ACh.; (4) and (5) differential record from both membranes—(4) 6 mg. ACh.; (5) 70γ adrenaline, all intravenous.

acetylcholine. Later in the experiment, after two further injections, each of 5 mg. of acetylcholine, had been given, two injections of 6 mg. each (in the absence of eserine) were given, the response of the membrane on the operated side being first recorded, and then, as soon as this had subsided, that of the normal membrane to the same dose. The records obtained are shown at 3, and measurements show maximal increases of tension of 3·15 g. (normal) and 4·2 g. (denervated), with a ratio of only 1 : 1·3. This decline of the ratio, with successive injections of acetylcholine, must indicate that the response to it of the ganglion cells on the denervated side is more readily depressed, since there is no corresponding fall in the responses to adrenaline. The point to be emphasized here is, that in all experiments recorded hitherto the augmentation ratio is never greater for the response to acetylcholine than for that to adrenaline; where there is any indication of a change in the reaction of the dener-

vated ganglion cells, it is in the direction of a diminished response to acetylcholine. This is further illustrated in tracings 4 and 5 of Fig. 5. These were taken about half an hour after the records shown at 3, and were made with simultaneous attachment of both nictitating membranes to the two ends of the differential torsion-lever. The tracings accordingly show the excess of tension developed by the denervated membrane over that of the normal membrane in response to further injections of 6 mg. of acetylcholine at 4, and 70γ of adrenaline at 5. It will be seen that, again, the excess of tension in the response to adrenaline, as shown by the deviation of the differential lever to the membrane of the operated side, is definitely greater than that in the response to acetylcholine.

The experiments so far described in this paper cover the whole range of doses of both adrenaline and acetylcholine, from the threshold responses of the normal nictitating membrane up to doses of acetylcholine large enough to cause depressant effects on the ganglion cells, which will be discussed in more detail in a later section. It seemed necessary, however, to examine further the effects of subthreshold doses, which produce reactions on the denervated side only, failing to excite the normal muscle cells of the nictitating membrane. From an inspection of our records we are able to confirm the finding of Cannon & Rosenblueth, as expressed by Fig. 1 of their paper, that such minimal doses of acetylcholine do often (though not always) produce somewhat greater effects on the denervated side, than those produced by threshold doses of adrenaline. Although, for reasons given earlier, we do not consider such reactions strictly comparable, because of the different modes of action of the peripheral transmitter in the two cases, the possibility of an enhanced reaction of the ganglion cells (perhaps due to the absence of cholinesterase) cannot be entirely excluded. In this respect experiments with direct application of acetylcholine to the surface of the ganglia seemed to offer a better chance for comparison than intravenous or arterial injections, because the reactions produced by direct application of acetylcholine to the ganglion are the result of a process of slow diffusion of the drug, possibly more comparable to the diffusion of adrenaline from the blood vessels to the muscle cells. As can be seen from Fig. 6, the time relations of the two reactions are, in fact, not dissimilar. In these experiments I was, however, frankly unable to reproduce the findings of Cannon & Rosenblueth. Fig. 6 represents the reactions of a normal nictitating membrane (lower line) and its denervated partner (upper line) to intravenous injections of adrenaline at 1, 2 and 3, the doses being 10, 20 and 40γ respectively, and to direct application of

different solutions of acetylcholine to the corresponding superior cervical ganglia at 4, 5 and 6, the concentrations being, in this case, 1, 0·5 and 0·25 p.c. respectively. It will be seen that, although the concentration of 1 p.c. applied at 4 is the same as that which was used by Cannon & Rosenblueth in the experiment illustrated by their Fig. 2, the result is widely different. The difference between the reactions of the normal and the denervated side, in my experiment, is certainly not greater than the difference between the responses to adrenaline; it is, on the contrary, considerably less pronounced. The same result was obtained with lower concentrations at 5 and 6. Altogether four experiments of this type were

Fig. 6. Effects of adrenaline intravenous and of ACh. directly applied to ganglia. Normal (lower) and preganglionically denervated (9 days) nictitating membranes (upper). (1) 10γ, (2) 20γ and (3) 40γ of adrenaline; (4) application of 1 p.c., (5) of 0·5 p.c. and (6) of 0·25 p.c. solutions of ACh. to both ganglia.

made, and in three of them the results were similar to those represented in Fig. 6. In one experiment, however, injections of doses of adrenaline varying from 2·5 to 20γ gave smaller reactions on the denervated side than on the normal side, and in this case, as was to be expected from the results of our previous experiments, local applications of acetylcholine to the ganglia also gave smaller responses on the denervated side. The evidence of my experiments with direct application of acetylcholine to the superior cervical ganglion is, therefore, not in favour of ganglionic sensitization. The fact that, in these experiments, the differences between the responses of the normal and the denervated side appeared to be less pronounced than with intravenous or arterial injections, may be due to an early depressant effect of acetylcholine on the superficial ganglion cells, first reached by this drug. This depression may also account for the quicker return of the operated nictitating membrane to a state of relaxation, as seen in reaction No. 4, in Fig. 6. It may be added that,

when I applied a 5 p.c. solution of KCl to the surfaces of the ganglia, with which Cannon & Rosenblueth obtained a large response from the denervated but none from the normal side, my result again differed from theirs, in that, in my experiments, such a solution always had a pronounced stimulating effect even on the normal ganglion.

(3) *Paralytic action of acetylcholine and effect of eserine*

Feldberg & Vartiainen [1934] showed that, with injections of acetylcholine into the circulation of a perfused ganglion, increase of the dose above a certain amount caused no further increase but, on the contrary, a curtailment of the mechanical response of the nictitating membrane, and therefore presumably of the output of impulses from the ganglion cells. This secondary, depressant effect of acetylcholine has already been mentioned in connexion with the experiment illustrated by Fig. 4, when it was noted that the denervated ganglion seemed to be more susceptible to it than the normal, control ganglion. This difference might presumably be associated with the loss of the special concentration of cholinesterase at the preganglionic synaptic endings, enabling acetylcholine to persist abnormally in contact with the ganglion cells, after the stimulating action due to its rapid appearance there. The experiments of Brown & Feldberg [1936] have shown that the chief effect of eserine, in delaying the disappearance from the synapses of acetylcholine liberated there by preganglionic impulses, is to depress the response of the ganglion cells. Whether for this reason, or from a directly paralytic effect of its own, eserine in more than a certain dosage completely paralyses the response of ganglion cells to preganglionic impulses, or to acetylcholine artificially applied [Feldberg & Vartiainen, 1934; cf. also Eccles, 1934]. Other experiments have confirmed and made more definite the contrast already mentioned, showing that the denervated ganglion is more susceptible to this delayed, depressant action of acetylcholine. The difference is seen more clearly with arterial injection into the innominate artery, and is greatly accentuated by eserine. Fig. 7 is a record from such an experiment, the upper line recording the contractions of the nictitating membrane on the side of preganglionic denervation, the lower line that of the normal side, in an atropinized cat, whose eyeballs had been removed. At 1 and 2 are seen the comparative effects of 0·2 mg. acetylcholine by arterial injection, and of 20γ adrenaline. It will be seen that the response on the normal side is rather greater than that on the denervated side. Between 2 and 3, 2 mg. of eserine were injected intravenously. 3 and 4 show the effects of two successive arterial in-

jections of 0·2 mg. acetylcholine. It will be seen that the first of this pair of injections, at 3, causes accentuated retraction of both membranes, but that the response on the denervated side is curtailed, and that the second injection, given about 1 min. later at 4, causes a further, large retraction of the normal membrane, but no effect at all on the denervated side. The response of the denervated ganglion is, for the time being, completely suppressed. 11 min. later a further pair of arterial injections, each of 0·2 mg. acetylcholine, was given at 5 and 6. The effect of the eserine is weakening, and there is now a response, though a diminished one, of the denervated ganglion to the second injection of the pair, at 6. A further injection of 1 mg. of eserine, between 6 and 7, restores the earlier condition, in that the denervated ganglion responds to 0·2 mg. of

Fig. 7. Paralytic effects of ACh. after eserine, more pronounced on the denervated side. *See text.*

acetylcholine at 7, but gives no response to a similar injection at 8. In this case, however, it will be seen that the injection at 7 causes some depression of the response, even of the normal ganglion, to the further injection at 8. Finally, to demonstrate that the effects recorded were due to the action of the acetylcholine injections on the ganglia, the normal ganglion was removed. A further arterial injection, at 9, then caused no movement of the membrane on that side, but that on the side of the original nerve section, where the denervated ganglion was still present, gave a reduced contraction, showing that this ganglion was slowly recovering from the complete paralysis demonstrated at 8.

It will be observed that the presence of eserine has two effects on the response to acetylcholine, which can be seen in the responses to each group of two rapidly consecutive injections, shown in Fig. 7. The responses at 3 and 5, and again at 7, are larger than those produced by the same injection of acetylcholine given before eserine at 1. This potentiation is seen on both sides, but is rather more pronounced on the normal side than on that of the denervated ganglion. This phenomenon has been

described earlier in this paper in connexion with Fig. 4, and is more conspicuous when the eyes are left intact and the original responses of the two nictitating membranes differ greatly in size, owing to the effect of peripheral sensitization. When we look at the responses to the second injections of the three pairs at 4, 6 and 8, on the other hand, it is seen that the secondary depressant effect of acetylcholine is much more strongly accentuated by eserine in the case of the denervated ganglion, than in that of the normal one. It seems reasonable to suggest that the potentiating effect of eserine on the primary, stimulant action is due to its weakening of the action of cholinesterase in the blood and tissues, which the acetylcholine has to encounter on its way to the ganglion cells, and which will affect equally the responses on the normal and on the denervated sides. It is, in effect, an increase of the dose of acetylcholine reaching the ganglion cells. The enhancement of the secondary, depressant action, on the other hand, which is so conspicuous on the denervated ganglion, must be connected with the defect of the cholinesterase concentration at the synapses, which normally protects the ganglion cells from the persistent effect of acetylcholine reaching them. It may be supposed that what remains of this local mechanism in the denervated ganglion is easily suppressed by eserine, while on the normal side it remains effective, and only shows signs of weakening after large doses of eserine.

Discussion

The experiments described in this paper give no support to the suggestion that denervation of the ganglion cells, by degeneration of the preganglionic nerve fibres, renders them more sensitive to the action of acetylcholine. We have seen that, whenever such preganglionic denervation is accompanied by an augmented retraction of the corresponding nictitating membrane in response to acetylcholine, the response to the peripheral action of adrenaline is similarly enhanced. When a quantitative comparison has been made, the tensions of the responses to adrenaline have been found to be augmented in a higher ratio than those of the responses to acetylcholine. This lack of evidence of ganglionic sensitization is, perhaps, surprising, in view of the remarkable sensitization of voluntary muscle fibres to acetylcholine, which accompanies degeneration of their motor nerve fibres. In both cases there is reason to suppose that cholinesterase is normally concentrated at the nerve endings, and that this concentration disappears with degeneration of the preganglionic or motor fibres. In the case of the preganglionic fibres I have given direct evidence of such disappearance. Direct evidence of a concentration of

cholinesterase at the motor nerve endings in voluntary muscle has recently been obtained by Marnay & Nachmansohn [1937]. It must be remembered, however, that a contrast of a somewhat similar kind has been observed between the effects, on voluntary muscle fibre and ganglion cells respectively, of depressing the action of this locally concentrated cholinesterase by eserine. Eserine causes the muscle fibre to give a repetitive, tetanic response to the abnormally persistent acetylcholine, liberated by a single motor nerve impulse, but there is no evidence, as yet, that eserine similarly causes the ganglion cell to give a repetitive output of postganglionic impulses, though Cannon & Rosenblueth (1937) have recently found that prostigmin, under appropriate conditions, may produce on the ganglion a relatively weak effect of this type. The evidence yet available suggests, in any case, that, if the acetylcholine liberated by a preganglionic impulse remains in contact with the ganglion cell, after it has caused the discharge of a postganglionic impulse, its effect is often to depress the response of the ganglion cell to further liberations of acetylcholine, rather than to stimulate it to repetitive activity. The meaning of this contrast between the two cases, as regards the relative prominence of the stimulating and depressant effects of the abnormally persisting acetylcholine, is by no means clear as yet. It may fairly be said, however, that, on the experimental facts as they are known, it is no matter for surprise that the loss, by preganglionic denervation, of the cholinesterase concentration at the ganglionic synapses, should accentuate the delayed depressant effects, rather than the immediate stimulant effect of acetylcholine on the ganglion cells. It would not, of course, be justifiable to suggest that the degeneration of the preganglionic fibres, and the consequent disappearance of the concentration of the cholinesterase from the neighbourhood of the synapses, would never, under any conditions, cause an enhanced response of the ganglion cells to acetylcholine, however applied. Conditions might indeed be found in which the degeneration would, for example, lower the minimal dose of acetylcholine, producing an effective stimulation of the ganglion cells. I am concerned only with the evidence for an increased response to acetylcholine, reaching the ganglion cells through the circulation or by local application, as afforded by the enhancement of a peripheral effect, such as retraction of the nictitating membrane. As to the existence of such enhanced effects, my experiments entirely confirm those of Cannon & Rosenblueth. I differ from them chiefly in interpretation. They regard the effects as evidence of sensitization of the ganglion cells to acetylcholine, whereas my evidence suggests that they are explicable by the sensitization of the

peripheral structures to the sympathetic transmitter, as demonstrated by their enhanced responses to adrenaline.

Summary

1. Preganglionic section, followed by degeneration, of the cervical sympathetic nerve usually, though not always, causes peripheral sensitization to adrenaline of the mechanism which retracts the nictitating membrane.

2. If such sensitization is present, it is abolished or greatly diminished by removal of the eyeballs.

3. The effects of injected acetylcholine on this mechanism run generally parallel with those of adrenaline, for doses of acetylcholine which are large enough to excite the ganglion cells of the normal ganglion, but not so great as to paralyse those of the denervated ganglion. For smaller doses they are equally parallel, if these doses are applied to the ganglion in such a way that the mode of action of the peripheral transmitter resembles that of injected adrenaline; that is, by local application to the surface of the ganglion.

4. The enhancement of the effects of acetylcholine by preganglionic denervation appears, accordingly, to be adequately explained by the peripheral sensitization to adrenaline and sympathin, without invoking an enhanced response to acetylcholine of the denervated ganglion cells.

5. The denervated ganglion is, however, much more easily paralysed by acetylcholine than the normal ganglion, and this can be explained by the diminution of esterase concentration on the denervated side.

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LIPOID SUBSTANCES OF THE OVARY DURING OVA PRODUCTION IN *RANA PIPIENS*

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NEEDHAM [1931] has developed the interesting hypothesis that embryos of terrestrial eggs utilize fat rather than carbohydrate as a source of energy because the oxidation of fats sets free relatively more water, and conservation of water is of vital importance in eggs developed on land. From the data of a number of authors, Needham [1931] has taken examples to show that eggs of domestic fowl, silkworms, lackey moths and grasshoppers burn during development between 54 and 85 p.c. of the available stores of fatty acids, while the aquatic embryos of frogs, plaice and sea-urchins burn from less than 10–28 p.c. of the fatty acids initially provided in the egg.

It was therefore surprising to find in the present investigation that during the production of ova there occurs a tremendous increase in the lipoid substance of the ovaries of frogs. If but a small portion of this fat is to be used for energy purposes by the embryo, then the rest must be used structurally if, indeed, it is used at all. The eggs of frogs have been previously reported to contain from 10 to 13 p.c. (wet weight) of fatty acids [Parnas & Krasinska, 1921; Terroine & Barthélemy, 1923; Fauré-Fremiet & Dragoiu, 1923]. Klenk [1933] reported that the composition of fatty acids in frogs resembled that of fresh-water fishes and lay between that of mammals and marine fishes. Many highly unsaturated fatty acids are present such as palmitoleic, linoleic, linolenic, arachidonic and clupanodonic acids and these impart a liquid consistency to the fat of the ovaries and give to it the high iodine number of 142 [Terroine *et al.* 1930].

In the present work, ovaries were removed from twenty-one freshly killed frogs of the species *Rana pipiens*. These animals had been kept in a frog pond at temperatures just above freezing during the winter months and they were killed for analysis in the spring. None appeared to

be diseased in any way. In fifteen of the frogs, the ovaries were found laden with ova and the combined weights of the two ovaries of such animals averaged well over 2 g. The weight of the ovaries in the remaining six animals was but a fraction of a gram. The average weight of the six frogs containing ovaries not loaded with ova was 20·0 ± 4·2 g. (mean ± standard deviation) and of the fifteen frogs containing ova-laden ovaries was 23·5 ± 4·5 g. The average weight of the ovaries in the latter group was 3·70 ± 0·98 g. and in the former group 0·14 ± 0·01 g. so that the differences in the weights of the entire animals was explained as the added weight of the ova-laden ovaries. Non-ova-laden ovaries were found to average 0·68 ± 0·06 p.c. of the weight of the whole frog and ova-laden ovaries 16·0 ± 2·9 p.c.

The frogs were double-pithed, the ovaries dissected out and weighed to three significant figures. They were then ground with cleaned sand, extracted with alcohol-ether and the extracts analysed by Boyd's modification of the oxidative micromethods of Bloor as used in previous studies [Boyd, 1936]. The results obtained have been reported in Table I as mg. of lipoid substance per 100 g. of ovary (combined weights, wet).

TABLE I. The lipoid composition of the ovaries of frogs

Weight of ovaries g.	Total lipoid substance mg. p.c.	Composition of total lipoid in mg. p.c.					
		Neutral fat	Total fatty acids	Cholesterol			Phospho-lipin
				Total	Ester	Free	
0·119	1,795	947	1,351	170	0	170	678
0·130	1,113	417	736	187	0	187	509
0·140	720	205	412	190	0	190	325
0·155	1,311	680	961	159	0	159	472
0·163	2,668	1,850	2,220	141	50	91	644
0·267	2,641	1,660	2,050	270	0	270	711
2·43	11,582	8,100	9,500	697	171	526	2570
2·51	10,350	6,670	8,200	1000	403	597	2410
2·97	12,053	6,950	9,320	1260	707	553	3370
3·22	10,687	7,650	8,800	727	0	727	2310
3·23	10,706	6,880	8,600	838	356	482	2750
3·33	11,768	8,200	9,500	1130	400	730	2170
3·35	8,175	5,680	6,770	430	22	398	2050
3·39	11,773	8,160	9,680	808	276	532	2620
3·67	11,456	7,280	9,200	954	437	517	2830
3·78	17,882	14,950	16,100	718	65	653	2170
3·82	11,566	8,300	9,600	738	131	607	2440
3·96	12,193	8,380	10,100	733	446	287	2780
4·23	9,336	6,700	7,620	904	406	498	1460
5·41	9,422	6,800	7,800	644	191	453	1850
6·35	6,892	4,800	5,420	930	410	520	888

The formation of ova was seen to be accompanied by a pronounced increase in the lipoid content of the ovaries. Compared with the *lowest* value in the first six animals of Table I, the maximal increases were:

total lipoid material, 2400 p.c.; neutral fat, 7000 p.c.; total fatty acids, 3800 p.c.; total cholesterol, 800 p.c.; free cholesterol, 700 p.c.; and phospholipin, 900 p.c. Compared with the *highest* value of the first six animals, the minimal increases were: total lipoid material, 158 p.c.; neutral fat, 160 p.c.; total fatty acids, 145 p.c.; total cholesterol, 59 p.c.; free cholesterol, 6 p.c.; and phospholipin, 25 p.c. Comparing the means of the first six animals with those of the remaining fifteen in Table I, the following average percentage increases were obtained:

Total lipoid substance...	546
Neutral fat	710
Total fatty acids	600
Total cholesterol	349
Ester cholesterol	(from practically none to an average of 295)
Free cholesterol	203
Phospholipin	316

The results demonstrate such a marked change in lipoid content that it appears superfluous to state that they were calculated to be statistically significant. However, the coefficient of correlation of one set of variables, weight and free cholesterol content of the ovaries, may be cited to illustrate the significance. Statistical formulæ used were those of Currie [1930]. Free cholesterol was chosen for citation because the increases in free cholesterol were less than those of any other lipoid substance and if the change in free cholesterol were found statistically significant then changes in the other lipoid substances must necessarily be similarly significant and more so. The coefficient of correlation and its probable error for the variables weight and free cholesterol of the ovaries were calculated to be $+0{\cdot}72 \pm 0{\cdot}07$. These figures indicate that the variables varied in the same direction with almost perfect correlation since the coefficient was close to 1 which is perfect correlation and that the odds were overwhelming in favour of its being significant since the coefficient of correlation was much greater than four times its probable error.

Summary

A statistically significant increase averaging from 200 to 700 p.c. was found by oxidative micromethods in the total lipoid substance, neutral fat, total fatty acids, total cholesterol, ester cholesterol, free cholesterol and phospholipin content of the ovaries of *Rana pipiens* during the production of ova.

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FACTORS AFFECTING THE SODIUM, POTASSIUM AND TOTAL BASE CONTENT OF THE ANTERIOR RETRACTOR OF THE BYSSUS OF *MYTILUS EDULIS*

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Smooth muscle contains less potassium and more sodium than skeletal muscle. A high sodium potassium ratio has been found by Saiki [1908] in pig's stomach; by Constantino [1911] in ox stomach, ox retractor penis, cow uterus and fowl stomach, the uterus being particularly low in potassium; by Meigs & Ryan [1912] in frog stomach; by Wilkins [1934] in cow uterus and bladder.

The following experiments were performed to determine the sodium potassium and the total base content of *Mytilus* muscle and to study the conditions affecting the passage of sodium and potassium ions into and out of the muscle.

Experimental

The muscles immediately on removal from the animal were lightly blotted with Whatman's filter paper, No. 42, and weighed into Pyrex tubes. Reweighing after 4 hr. heating at 110° C. yielded the water content. The total solids were determined by ashing the muscle with sulphuric-nitric acid mixture and then weighing. Sodium was estimated in the ash by Eisenmann's [1927] modification of the titrometric method of Kramer and Gittleman for the micro-determination of sodium. In control experiments, if the total sodium in the solution exceeded 0·3 mg., an accuracy of ±1 p.c. was obtained. Below this amount the results were too low; with 0·2 mg. the results were 30 p.c. too low, and with 0·1 mg. about 50 p.c. too low. Potassium was estimated by Briggs' [1923] diazo cobalti-nitrite micro-colorimetric method with certain modifications. On addition of sodium silver cobalti-nitrite to the solution, the resulting mixture was allowed to stand for 16 hr. instead of 2 hr. and 33 p.c. acetic acid was used for dilutions instead of water, as by this method the resulting mixture was found to be more stable.

Experiments with known amounts of potassium showed that 0·1–0·5 mg. could be determined with an accuracy of ±1 p.c. If the sodium and potassium were to be determined in the same muscle, the ash was dissolved in 4 c.c. of distilled water, 2 c.c. was removed for the determination of potassium, and the rest was evaporated to 1 c.c. and the sodium determined. To ensure that the absolute amounts of sodium and potassium determined exceeded 0·4 and 0·1 mg. respectively, more than one muscle was sometimes used.

Total base was determined by the method of electrodialysis [Adair & Keys, 1934] for the micro-determination of base in the blood. In place of blood the muscle was used unminced in 2 c.c. of distilled water. If the muscle had been soaked in *Mytilus* saline, an hour was usually sufficient for electrodialysis, but muscles that had swelled greatly in certain solutions were electrodialysed for a longer period (4–6 hr.); muscles soaked in solutions of lithium chloride, calcium chloride, sodium iodide and sodium thiocyanate were difficult to be electrodialysed, so that the period of analysis exceeded 12 hr. To minimize the difficulty the contents of the anode vessel were stirred with a glass rod during electrodialysis of muscles soaked in sodium iodide and sodium thiocyanate, the amount of mercury used being greater than normal. Muscles soaked in magnesium chloride could not be electrodialysed. In control experiments with known amounts of chloride, 98–99 p.c. of lithium, sodium, ammonium and potassium, 90–96 p.c. of calcium, strontium, and barium were recovered.

The composition of *Mytilus* saline was approximately that of sea water with the omission of magnesium. It was prepared as follows: 1·8 c.c. of 0·564 *M* KCl, 2·8 c.c. of 0·376 *M* $CaCl_2$, 5 c.c. of *M*/15 sodium phosphate at *p*H 7·2 with requisite amount of NaCl to render the buffer isotonic, and the total volume brought up to 100 c.c. by adding 0·564 *M* NaCl.

Results

The mean sodium content of twenty-six freshly dissected muscles was 6·6 ± 1·6 mg./g. of wet muscle. The sodium content of the right and left retractors was closely similar, the mean difference between the values for thirteen pairs being 5·3 ± 5·1 p.c. and the maximum difference being 17·2 p.c. The sodium content of muscles from different animals varied over a wide range from 4·4 to 9·5 mg./g. Generally the sodium content of muscles from animals freshly arrived from Plymouth was relatively low. In six such muscles the mean value was only 4·5 ± 0·3 mg./g. Higher values were found when mussels had been stored in the refrigerator

for 2 or 3 weeks at a temperature of about 5° C. Four such muscles contained as much as 7·2 ± 0·15 mg./g. Analyses of the chloride content of both muscles and blood were made by Dr. M. G. Eggleton after 17 days, and 24 days' storage of the animals in the refrigerator. The blood appeared to become more concentrated, its NaCl rising from 3·7 p.c. at 10 days to 4–4·5 p.c. at 24 days. The chloride content of the muscle showed a relatively greater increase, so that the muscles Cl: blood Cl ratio, which at 10 days was 0·2–0·3, at 17 days was 0·3–0·36, and at 24 days 0·4–0·5.

Six freshly dissected muscles contained 1·4 ± 0·15 mg./g. potassium and 3·8 ± 0·9 mg./g. sodium. After soaking in *Mytilus* saline for 4 hr., six muscles from animals belonging to the same batch contained 0·9 ± 0·14 mg. potassium and 5·0 ± 0·2 mg. sodium. The potassium content was therefore less and the sodium content greater after soaking the muscle for 4 hr. The potassium appears to have diffused out from the fibres as it does from frog muscle [Fenn & Cobb, 1934; Fenn, 1936]. The average ratio of sodium to potassium content of the freshly dissected muscles was about 2·2 : 1. This contrasts with that of 1 : 16 in skeletal muscle [Fenn & Cobb, 1934; Mond & Netter, 1932].

The sodium content of the above muscles before soaking was 31 p.c. of that of the *Mytilus* saline; after soaking it was 41 p.c. The chloride content of two muscles (analyses by M. G. Eggleton) was 24 p.c. before soaking and 40 p.c. after soaking for 2 hr. The chloride analyses are too few, but they suggest that as in the frog sartorius [Fenn *et al.* 1934] the sodium content is initially greater than the chloride content, and that after soaking the two nearly become equal.

The total sodium and potassium content was only from 37 to 52 p.c. of the expected cationic content calculated on the assumption that the muscle was isotonic with sea water. To determine whether a metallic cation was responsible for the deficit, the total cations were determined by treating with sulphuric acid and ashing. The mean weight of the ash in six determinations was 16·2 ± 0·9 mg./g., but values up to 25 mg./g. were found. The results were checked by electrodialysis which gave a mean value (seven determinations) of 0·242 ± 0·009 m.eq./g. If the ash is expressed as cation sulphates, the mean value of the above determinations is 0·236 ± 0·011 m.eq. The total sodium and potassium content estimated in the six freshly dissected muscles mentioned above is equivalent to 0·205 ± 0·028 m.eq./g. Electrodialysis measures the total diffusible base, both organic and inorganic. Traces of trimethylamine were recognized in the dialysate by smell on addition of alkali. As the figures of electrodialysis include sodium, potassium, calcium, magnesium and any other

base that might be present, they are somewhat higher than the figures showing sodium and potassium alone. The relatively small differences between the values of the combined sodium and potassium content on the one hand and the electrodialysable base and the weight of ash on the other, indicates that sodium and potassium account for the greater part of the inorganic constituents of the muscle.

As mentioned above, *Mytilus* saline, like sea water, was equivalent to 0·564 m.eq./c.c. solution. To compare the osmotic relations between the muscle content and its surrounding medium, the water content of muscle must be known. In twenty-two muscles water accounted for 78 ± 2 p.c. of the weight of the muscle, and the total cationic concentration in the muscle should be therefore 0·44 m.eq./g. if the muscle contents are in osmotic equilibrium with the surrounding solution. In fact, the muscle salt content, as shown above, is only about one-half as concentrated as this, although such muscles played in *Mytilus* saline do not change weight. The total base in *Mytilus* blood (three analyses) and in cœlomic fluid (three analyses) was $0{\cdot}523 \pm 0{\cdot}026$ m.eq./c.c. (animals stored for 5 days), approximately the same as that of sea water. The chloride content of blood and cœlomic fluid (three analyses by M. G. Eggleton) was that of 3·7 p.c. NaCl or equivalent to 0·632 m.eq./c.c. of base (animals stored for 10 days). The osmotic pressure of the blood and cœlomic fluid by the vapour-pressure method (two observations by J. R. Pappenheimer from the same batch of animals stored for 8 days) was that of 0·584 *M* NaCl or 0·584 m.eq./c.c. of base; the osmotic pressure of sea water was that of 0·552 m.eq./c.c. of base. The total base figures are less than the figures from vapour-pressure determinations, as only about 98 p.c. of sodium and potassium are recovered and the recovery of magnesium is incomplete. Correcting for this deficiency and adding values for the magnesium content (0·04 m.eq.), the total base in the blood becomes 0·585 m.eq./c.c. Previous analysis of *Mytilus edulis* tissues show the total ash to be 1·3 p.c. of the wet tissue [Balland, 1898], soluble salts in smooth muscle to be 1–1·2 p.c. [Frederic, 1901]; these figures are all about 50 p.c. of the expected values. The molecular concentration, is, however the same as that of the external medium, the deficiency in salt content being made up by some organic substance (taurine, suggested by Frederic [1901]); the depression of the freezing-point of blood of *Mytilus edulis* is 2·26° C., that of the external sea water being 2·11–2·14° C. [Monti, 1914].

As with the sodium content, so with the total base, wide variations were found in the analysis of freshly dissected muscles. The mean value

of another eight determinations was 0·295 m.eq., the maximum value being 0·351 m.eq. These muscles were from animals that had been stored for days in the refrigerator, and the values are higher than those, 0·242 ± 0·009 m.eq., mentioned above from animals newly arrived.

It would be expected that the total base might increase if the muscle was placed in *Mytilus* saline owing to diffusion of ions into the muscle and possible diffusion outwards of the organic substance. This did not always happen. The total base sometimes increased and sometimes decreased. But generally the total base was greater and of more constant value if the muscles had been soaked in *Mytilus* saline for 16 hr. In ten muscles so treated, the total base varied from 0·292 to 0·401 m.eq./g., the mean value being 0·337 m.eq./g., the weights of the muscles after soaking being within 3 p.c. of their initial weights. The total base at the end of the period accounted for 65–89 p.c. of the osmotic pressure, the mean value being 75 p.c. The total base might increase appreciably even in 1 hr.; in thirteen muscles so treated the total base varied from 0·271 to 0·351 m.eq., the mean value being 0·292 ± 0·030 m.eq. The mean total base content of three muscles soaked for 2½ hr. in *Mytilus* saline containing twice the usual calcium content (0·02 *M* $CaCl_2$) was 0·305 ± 0·008 m.eq.; the mean total base content of the corresponding three muscles, killed by immersion in the same *Mytilus* saline at 40–45° C. for half an hour and further soaking at room temperature for 2 hr., was 0·467 ± 0·007 m.eq., the salt content being approximately the same as that of sea water, allowing for the water content of the muscle. The organic substance responsible for part of the osmotic pressure is therefore, like potassium, relatively indiffusible.

The total base in the muscle is also affected by varying the temperature at which the animals have been stored. Muscles were analysed directly after removal from animals that had been stored in the refrigerator at a temperature of about 5° C. for about 24 hr. after arrival from Plymouth. The mean value of nine estimations was 0·227 ± 0·019 m.eq. The mean value of the base content of eight muscles dissected from animals that had been kept at a lower temperature, so that some of the sea water was frozen, was 0·311 ± 0·44 m.eq., the maximum value being 0·425 m.eq. Some muscles from this cooled group were removed and kept at room temperature for 6 hr. The mean value of the total base in six muscles of this group was 0·224 ± 0·019 m.eq. It will be seen that the total base in muscles from animals kept at lower temperature was higher than that in muscles from animals kept at a higher temperature. It is possible that this may be due to hypertonicity of sea water remaining after some

of the water had frozen out. These experiments show that the base concentration of muscles may change *in vivo*. Seasonal changes in the sodium, potassium and calcium content have been found in frog muscle [Mond & Netter, 1932].

Effect of immersion in solutions of certain ions on weight and total base content

Isotonic solutions (0·564 *M*) of certain monovalent cations cause the muscle to gain weight and base in the order $LiCl < NaCl < NH_4Cl < KCl$ (Table I); this order is the same as that for the apparent ionic mobilities

TABLE I. Comparison of effects on weight and content of total base of immersion of muscle for 16 hr. in isotonic solutions of chlorides of certain cations and sodium salts of certain anions. Each value represents the mean of operations on six muscles. Solutions of monovalent ions were buffered with *M*/300 phosphate *p*H 7·2; solutions of divalent ions were unbuffered. The weights indicate the differences in weight of a muscle before and after immersion expressed as a percentage of the initial weight of the muscle. The total base is calculated as m.eq. per g. of the muscle before immersion.

A. *Weight increase*

Cations						
Li	Na	NH_4	K	Ca	Sr	Ba
20 ±17	31 ±14	44 ± 1	130 ±22	−6 ± 4	16 ± 4	−5 ± 6
Anions						
Cl	Br	NO_3		I	CNS	CN
20 ± 6	37 ± 9	51 ±17		69 ± 7	92 ±16	170 ±12

B. *Base increase*

Cations						
Li	Na	NH_4	K	Ca	Sr	Ba
0·265 ±0·91	0·476 ±0·029	0·582 ±0·076	1·040 ±0·100	0·149 ±0·07	0·254 ±0·02	0·420 ±0·060
Anions						
Cl	Br	NO_3		I	CNS	CN
0·436 ±0·060	0·521 ±0·04	0·650 ±0·07		0·742 ±0·04	0·707 ±0·104	1·390 ±0·160

of these cations in protoplasm [Osterhout, 1931], for their action in favouring ciliary movement in *Mytilus edulis* and other marine animals [Lillie, 1906], and with the exception of ammonium, the same as that for their rate of penetration into cells of *Valonia* [Osterhout *et al.* 1933]. Small concentrations of calcium and magnesium (0·01–0·02 *M* $CaCl_2$, $MgCl_2$) partly counteract the swelling and gain of base produced by the above salts. Small concentrations of potassium (0·01–0·02 *M* KCl) and increase in hydrogen-ion concentration (experimental range, *p*H 7·8–4·4)

partly or wholly counteract the swelling and gain of base produced by sodium chloride; swelling of muscle produced by 0·564 *M* NaCl in the absence of calcium is reversed when the latter is reintroduced (Fig. 2) or if the concentration of hydrogen ion is increased (*p*H 7·8–4·4). This action of hydrogen ion on *Mytilus* muscle is comparable to the action of hydrogen ion on the penetration of potassium into frog sartorius [Fenn & Cobb, 1934] and *Thyone* muscle [Steinbach, 1937].

Isotonic solutions of certain monovalent anions cause the muscle to gain weight and base in the order

$$NaCl < NaBr < NaNO_3 < NaI < NaSCN < NaCN.$$

The action of NaBr and $NaNO_3$ is approximately the same; out of six muscles $NaNO_3$ produced greater swelling and gain of base than NaBr in four, and less swelling and gain of base in two muscles; these anions cause swelling of the ciliated cells of gills of *Mytilus edulis* in the order $NaCl < NaNO_3 < NaBr < NaI < NaSCN$ [Lillie, 1906].

Increase of total base produced by sodium chloride and potassium chloride in the presence of 0·01 *M* $CaCl_2$ was about 30 p.c. less in summer than in winter. In summer the weight of muscles either decreased or remained stationary when immersed in isotonic *Mytilus* saline; in winter the weight increased. The above experiments were performed at room temperature (18–20° C. in winter, and 22–24° C. in summer): these variations, however, are not due to this slight difference in temperature, as increase in temperature increases the swelling [Meigs, 1912].

Isotonic solutions (0·376 *M*) of certain divalent cations cause the muscle to gain base in the order $CaCl_2 < SrCl_2 < BaCl_2$. Strontium chloride causes a greater swelling than calcium chloride, but the swelling in barium chloride is less than that in strontium chloride; when immersed in these solutions, the muscle contracts and at first loses and then gains weight (Fig. 1). The contraction is extreme in barium and is accompanied by shrinkage in length to 15–20 p.c. of its extended value and by loss of weight by about 15 p.c. within 10–20 min. The loss of weight appears to be due to contraction, and the contracted muscle gains weight more rapidly in barium chloride than in calcium chloride, so that the discrepancy between the gain of weight and gain of base in these solutions is only an apparent one (Fig. 1). Small concentrations of calcium (0·01 *M* $CaCl_2$), potassium (0·01 *M* KCl), and magnesium (0·02 *M* $MgCl_2$) partly counteract the effect of barium in causing increase in weight and base.

Mytilus muscle swells in hypotonic *Mytilus* saline and also swells in hypertonic saline after a preliminary loss due to exosmosis (Fig. 2). If

the concentration of calcium in the *Mytilus* saline is twice that of sea water or the same as that of blood (0·02 *M* $CaCl_2$), the muscle shrinks by about 15–20 p.c. of its original weight if the osmotic pressure of the saline is doubled by adding sodium chloride; it begins to swell after about 2 hr. If the concentration of calcium is the same as that of sea water or *Mytilus* saline, the muscle loses about 5–10 p.c. of its initial weight in 20 min. in the hypertonic saline and thereafter begins to swell; in the

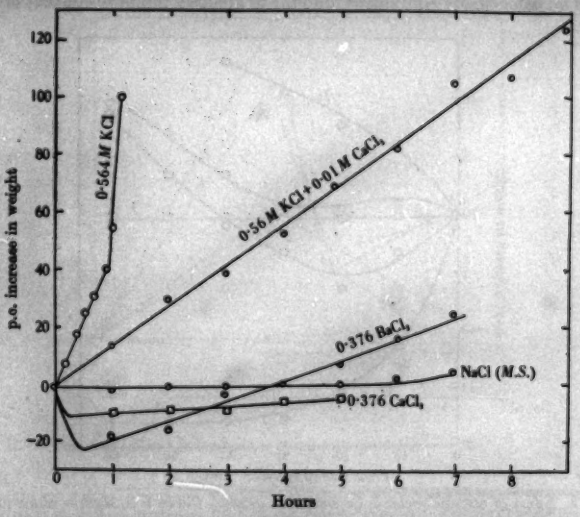

Fig. 1. Effect of certain cations on weight of muscle. *M.S.* = *Mytilus* saline.

absence of calcium the muscle swells without losing weight. The adductor muscle of clam also swells in hypertonic sea water [Meigs, 1914]. The swelling of *Mytilus* muscle is accentuated by deficiency of calcium, and is therefore due to the action of excess sodium comparable to the action of excess ammonium or potassium. The increase in weight in hypertonic *Mytilus* saline is comparable to the increase in weight of some muscles in isotonic *Mytilus* saline, of frog smooth striated muscle in isotonic Ringer [Meigs, 1912], of *Mytilus* muscle in isotonic sodium, potassium and ammonium chlorides, and of frog muscle in hypertonic potassium chloride [v. Fürth, 1919]. The action of excess sodium thus resembles the action of excess potassium, the monovalent cations Li, Na, NH_4, K

all causing an increase in weight. Frog smooth muscle swells in hypotonic and loses weight in hypertonic Ringer [Meigs, 1912; Evans, 1926]. The swelling of *Mytilus* and clam muscles in hypertonic solutions is probably due to the fact that the sodium chloride content of *Mytilus* saline or sea water is very high (3 p.c. compared with 0·7 p.c. of frog Ringer).

If the sodium chloride of the *Mytilus* saline is reduced by replacement with an osmotically equivalent amount of glucose, the base content of the

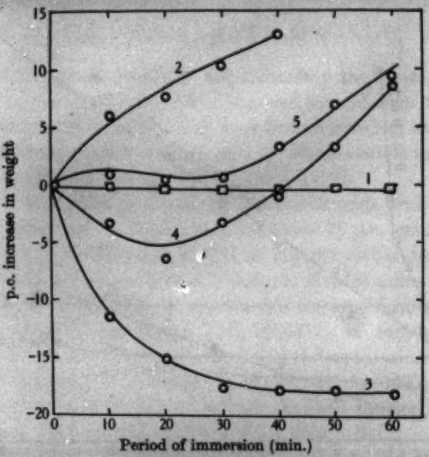

Fig. 2. Effect of osmotic pressure on weight of muscle. Curve 1 in *Mytilus* saline (0·01–0·02 *M* $CaCl_2$). Curve 2 in *Mytilus* saline, NaCl content and osmotic pressure both half the normal. Curve 3 in *Mytilus* saline, with calcium, NaCl content and osmotic pressure all twice the normal, the calcium content being that of blood. Curve 4 in *Mytilus* saline with NaCl content and osmotic pressure twice the normal. Curve 5 in 1·128 *M* NaCl (osmotic pressure twice normal).

muscle is proportionately reduced. If, however, the sodium chloride in the saline is reduced without osmotic replacement, the total base content of the muscle diminishes more than proportionately. Similarly when the muscle is placed in a hypertonic saline containing excess of sodium the increase in concentration of the base is greater than the corresponding increase in the saline. Thus the increase in weight of three muscles immersed for 16 hr. in *Mytilus* saline was 4 ± 9 p.c. and the concentration of base was $0{\cdot}335 \pm 0{\cdot}021$ m.eq./g.; increase in weight of the corre-

sponding three muscles in *Mytilus* saline, the osmotic pressure of which was reduced to 60 p.c. of normal by reducing the sodium chloride content, was 32·4 ± 6 p.c.; the concentration of base was 0·135 ± 0·042 m.eq./g., a reduction to 40 p.c. The increase in weight of another three muscles immersed in *Mytilus* saline was 6 ± 6 p.c., the concentration of base was 0·305 ± 0·027 m.eq./g. The increase in weight of the corresponding three muscles in *Mytilus* saline, the osmotic pressure of which was increased by

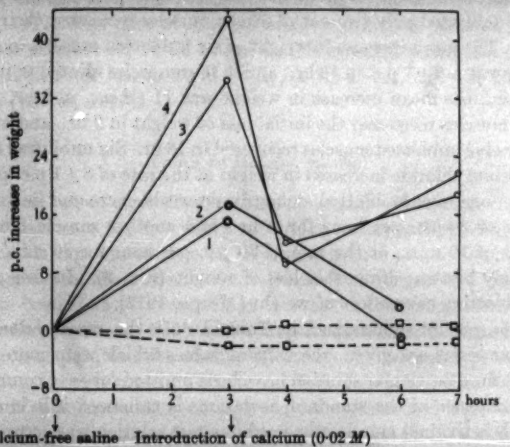

Fig. 3. Effect of blotting on loss of weight produced by calcium. The muscles were blotted at each point. They gained weight in calcium-free saline and lost weight when calcium (0·02 *M*) was reintroduced. The first two muscles (total experiments 6) which had been left undisturbed lost all weight they had gained, but the 3rd and 4th muscles (total experiments 4), blotted 1 hr. after immersion in 0·02 *M* $CaCl_2$ saline, gained weight after 3rd blotting. Weight of two control muscles in 0·02 *M* $CaCl_2$ saline remained stationary (broken lines).

40 p.c. by adding sodium chloride, was 21 ± 11 p.c.; the concentration of base was 0·472 ± 0·044 m.eq./g., an increase of 55 p.c.; this finding is comparable to the effect of sodium chloride on frog sartorius [Fenn *et al.* 1934].

Mechanical friction increases the swelling produced by various solutions. Muscles gain weight more rapidly when they are taken out and blotted (every 10 or 15 min.) than when they are merely immersed with slight aeration in solutions of potassium, sodium and barium

chlorides. Muscles immersed in 0·376 *M* $BaCl_2$ first lose weight and this loss of weight persists if they are undisturbed, so that after 16 hr. immersion, three muscles weighed 6 ± 1 p.c. less than their initial weight. If however muscles were removed from the solution and blotted at intervals of 1 hr. for 7 hr., there was a subsequent increase in weight amounting to $38{\cdot}4 \pm 13$ p.c. (three muscles after 18 hr.). The initial loss in weight is less and lasts for about 1 hr. if muscles are blotted as often as 10 min.; if blotted every hour the loss of weight persists for 4 or 5 hr., while if unblotted only two out of fifteen muscles recovered their weight in 16 hr. The mean increase in weight of six unblotted muscles in calcium chloride was -8 ± 7 p.c. in 16 hr.; and in four muscles blotted at intervals of 20 min., the mean increase in weight was 11 ± 8 p.c. in 2 hr. All the blotted muscles recovered the initial loss of weight in 2 hr., and only two out of twelve unblotted muscles recovered in 16 hr. Six unblotted muscles in potassium chloride increased in weight at the rate of 8 ± 1 p.c. per hour in 16 hr., one muscle, blotted at hourly intervals, increased in weight at the rate of 30 p.c. per hour for 9 hr., and another muscle blotted at intervals of 10 min., at the rate of 80 p.c. per hour for 80 min. Correspondingly blotting diminishes loss of weight (Fig. 3). In frog smooth muscle blotting causes loss of weight [Meigs, 1912].

In the results summarized in Table I only the mean values for a particular series are given, the control values which were more or less constant for a particular solution have been omitted for easy comparison. The significance of the standard deviations is enhanced if it is realized that each individual muscle immersed in a test solution was accompanied by a corresponding muscle in a control solution, and the results were so uniform that a difference between means shown in the table invariably represents qualitatively a corresponding difference in the values for the pairs of individual muscles.

The irritability of the muscles after 16 hr. immersion in these abnormal solutions was not tested, but the muscles in most solutions showed a deficit in total base compared to the base content of the external medium; if the muscles were dead, their base content would become the same as that of the saline in which they were soaked. The total base content of muscles soaked in potassium chloride, sodium cyanide, that of some muscles in sodium iodide and sodium thiocyanate, and of one out of twelve muscles soaked in sodium nitrate approached that of the external solutions. The electrodialysis of muscles soaked in sodium iodide and sodium thiocyanate was probably incomplete. In the presence of calcium (0·01 *M* $CaCl_2$) the concentration of base in six muscles soaked in

potassium chloride for 7 hr. was only 0·380–0·391 m.eq./g., and that in six muscles soaked in thiocyanate for 16 hr. was 0·336–0·339 m.eq./g. The muscle gains weight in some of the solutions in a shorter time (1–3 hr.) in the same order as it gains weight in 16 hr. (Figs. 1 and 3).

The adductor muscle of clam retains its irritability in half strength and double strength sea water for 24 hr. [Meigs, 1914]; frog stomach recovers its irritability after 24 hr. immersion in isotonic potassium chloride, though it swells during the time of immersion by 45–55 p.c. [Meigs, 1912]. The cilia of *Mytilus edulis* continue to beat in isotonic potassium chloride for 14–24 hr., in double strength potassium chloride (1·12*M* KCl) for 1 hr., in 0·4*M* $CaCl_2$ for 30–33 hr., in 0·4*M* $BaCl_2$ for 7–14 hr. [Lillie, 1906].

Tonus in isolated muscle

The three sets of conditions, soaking muscles in saline, removing them from animals kept for 2–3 weeks in a refrigerator, and removing them from animals kept unusually cold, have thus been shown to result usually in an increase in the content of total base. The former two conditions have also been shown to result in an increase in the sodium content. In these same conditions, the muscles developed a high tone and a high viscosity, comparable with similar effects on the retractor penis of the dog during "Ringer tonus" [Winton, 1930]. This parallelism suggests a significant correlation between high sodium content and high tone. The increase of tone was more marked if the *p*H was 7·8 (borate buffer) than it was at a *p*H below this value.

This excessive tone of muscles that had been obtained from animals long stored in the refrigerator, or had been subjected to unusually low temperatures, could be reduced if the muscles were placed in sodium deficient solutions, for example by replacing up to 30–40 p.c. of the sodium chloride of the *Mytilus* saline by an osmotically equivalent amount of glucose, or by treating the muscle with hypotonic saline, the osmotic pressure of which was 60–70 p.c. of the normal. The sodium content of *Mytilus* muscle was likewise reduced when treated with sodium deficient solutions, but figures are not quoted as the volume of inter-fibrillar spaces was not known, so that actual deficiency in the fibres could not be calculated. Tone was, however, reduced if the muscle was treated with a potassium rich solution, such as *Mytilus* saline, the sodium (80–100 p.c.) of which had been replaced with potassium, the effect being reversible.

Calcium and magnesium in concentration present in sea water decreased tone, so that one of the methods of diminishing the excessive tone of the above muscles was to place them for a couple of hours in sea water. This was also accompanied by loss of weight and diminution of base content. If the calcium content of the *Mytilus* saline was decreased, tone increased so that in calcium-free solutions the muscle was tonically contracted. This effect was more marked in winter than in summer muscles. The sodium content of the muscle was increased if it was placed in a calcium-free saline. The mean sodium content of the six right retractors soaked in *Mytilus* saline for 4 hr. was $5 \pm 0{\cdot}4$ mg./g.; the mean

TABLE II. Effect on the content of total base of soaking muscles (*a*) in *Mytilus* saline, (*b*) in calcium-free saline for 16 hr.; *p*H 7·2, phosphate buffered.

Mytilus saline m.eq./g.	Calcium-free saline m.eq./g.	p.c. increase in base in calcium-free solution
0·312	0·697	123
0·286	0·875	209
0·290	0·628	118
0·310	0·722	133
0·295	0·450	59
0·351	0·791	126

sodium content of the corresponding left retractors soaked in calcium-free saline was $6{\cdot}7 \pm 0{\cdot}02$ mg./g. Muscles thus soaked in calcium-free saline lose their tone when restored to normal *Mytilus* saline and will then contract to electrical stimulation. Similar effects are shown by estimation of the total base (Table II).

DISCUSSION

Two factors suggest the existence of a membrane round *Mytilus* plain muscle fibres: first, the swelling in hypotonic saline and shrinkage in hypertonic saline (Fig. 3); secondly, the fact that in living muscle part of the osmotic pressure is made up by an organic substance which is relatively indiffusible, and so appears to be contained in enclosed regions, just as potassium, and certain organic substances appear to be in frog sartorius muscle.

One of the differences between an isolated unstriated muscle and an isolated striated muscle is that the former is generally found in a state of contracture or tone. This may be due in part to the greater sodium and lesser potassium content of unstriated muscle. It is significant to note that the uterus which is capable of sustained tonic contraction, known as retraction, is particularly rich in sodium and poor in potassium [Constantino, 1911; Wilkins, 1934].

Summary

1. The large variations observed in the sodium and chloride content of *Mytilus* unstriated muscle are associated with length of storage of the animals, those from fresh muscles showing the lowest values.

2. Soaking such fresh muscles in *Mytilus* saline resulted in an increase in sodium and chloride content and decrease in potassium content.

3. The total base content of *Mytilus* unstriated muscle is only 50–70 p.c. of that present in sea water; the total base in *Mytilus* blood, cœlomic fluid and dead muscles soaked in *Mytilus* saline is approximately the same as that of sea water. The osmotic pressure of *Mytilus* blood is about the same as that of sea water.

4. When the whole animal had been at a low temperature surrounded by partly frozen sea water, the total base content of the muscle increased. This process was reversed when the animal was replaced in air at 18° C.

5. Immersion in solutions of certain monovalent ions causes muscle to gain weight and base in the following order:

$$Li < Na < NH_4 < K,$$
$$Cl < Br < NO_3 < I < CNS < CN.$$

6. Immersion in solutions of certain divalent cations causes the muscle to gain base in the order $Ca < Sr < Ba$.

7. Mechanical friction increases the gain in weight produced by the above ions.

8. Small concentrations of calcium, magnesium, potassium and decrease in *p*H partly antagonizes the effect of some of the above cations in causing increase in weight and base.

9. The tone of *Mytilus* muscle greatly increases under those conditions in which total base and sodium content of the muscle increases.

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EXCLUSION OF THE LIVER IN THE RABBIT

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The initial steps towards the present precise knowledge of the effect of hepatectomy in mammals were taken by F. C. Mann [1921, 1927] when he described a method for complete removal of the liver from dogs. This original method has since been simplified [Firor & Stimson, 1929; Markowitz *et al.* 1933], but it still remains an operative procedure too severe for smaller mammals such as the rabbit. In 1929 McMasters & Drury reported a method for hepatectomy in the rabbit. This is a simple one-stage operation, results in the removal of only 90–94 p.c. of the total liver tissue, and is consequently open to the serious objection that in experiments concerned with carbohydrate metabolism the remaining 10–6 p.c. of the liver can produce glucose at a sufficient rate to modify significantly the experimental result. Drury [1929] also described a two-stage method for the removal of the whole liver in which at the first operation there is established a collateral circulation to carry the blood normally flowing through the inferior vena cava and the portal vein, whilst at the second operation the liver is removed after ligating its afferent vessels and removing the upper portion of the inferior vena cava. In my hands this method has proved unsatisfactory in that the severity of the operative procedures required in the second operation produced such a state of traumatic shock that no satisfactory preparations were obtained.

These modern methods of hepatectomy all involve operations under an anæsthetic and afterwards a period of 2 or more hours for recovery. Then observations must be commenced as soon as the animal appears to the operator to be in a satisfactory condition. The experiments are thus, in a sense, acute experiments, and as such they are open to the objection that they are carried out on animals that have recently been anæsthetized

and recently traumatized. The profound effect of anæsthesia on carbohydrate metabolism is now well recognized [Evans *et al*, 1931; Murphy & Young, 1932], but the equally profound effect of operative trauma has as yet received little attention. The results reported in this paper show that after hepatectomy the effects of anæsthesia and operative trauma persist throughout the survival period of the animal, and materially influence the observations and conclusions relative to carbohydrate metabolism.

In the course of an investigation into the relationship of the liver to carbohydrate metabolism it became desirable to study hepatectomized preparations in which the interference of trauma and anæsthesia could be eliminated. A method was, therefore, devised by which at the time of the experiment the liver could be instantaneously and painlessly excluded from the circulation in an unanæsthetized animal. This method involves two preliminary operations. At the first, the portal circulation is impeded so that a collateral circulation develops. At the second, loose ligatures are placed around the vessels leading to and from the liver and the ends of these are brought out through the skin of the closed abdominal wall. Four days later when the animal has recovered from the second operation the liver can be cut out of the circulation in a few seconds by simply tightening the ligatures. This manipulation, judging from the animal's behaviour, is painless. This was anticipated, for it was found that, when only the abdominal wall of the rabbit was anæsthetized with novocaine, the unanæsthetized liver could be tied off without attracting the animal's attention, and secondly, that the drawing of thread through the human skin, as for example, in the removal of stitches, is painless.

The new method will first be described and then, by means of the results it yielded, it will be compared with those methods involving operation under anæsthesia some few hours before the commencement of observations.

Type of animal. Male rabbits were used. Their weight was approximately 2 kg. Animals of lower weight than this are too small to permit easy operating. Larger animals usually have too much intra-abdominal fat to allow rapid identification of the anatomical structures involved.

Preparation for operation and anæsthesia. The animals are deprived of food for 18 hr. before operation. Half an hour before the administration of the anæsthetic atropine sulphate gr. 1/100 is given subcutaneously. This prevents secretion of mucus during anæsthesia and thus lessens the risk of post-operative broncho-pneumonia. The anæsthetic used is ether administered in the usual cone mask. Oxygen is given if cyanosis develops.

Anæsthesia is first induced to a depth sufficient to abolish the eye reflex; after the abdomen is opened the anæsthesia is lightened till the eye reflex just returns; after closure of the peritoneum the anæsthetic is removed.

The skin of the abdominal wall is shaved from $\frac{1}{2}$ in. above the costal margin to the pelvis, and swabbed first with spirit and then with tincture of iodine.

General technique. The strictest asepsis must be observed. The animal should be completely covered with sterile sheets, and after the incision has been made sterile cloths should be clipped to the edges of the wound.

It is imperative to avoid the formation of adhesions. The minimum of intra-abdominal manipulation should be allowed and this should be gentle and deft. It is an advantage if the operator and his assistants wear smooth rubber gloves and keep these moistened with saline.

All ligatures are soaked in saline before use.

Two special instruments are required: a pair of angular nasal dressing forceps (Tilley's forceps); a piece of glass rod 2 mm. in diameter and 10 cm. long the terminal centimetre of which is bent at an angle of 45° to the shaft.

First operation (Fig. 1)

This operation is essentially the same as the first operation of the two-stage hepatectomy described by Drury [1929].

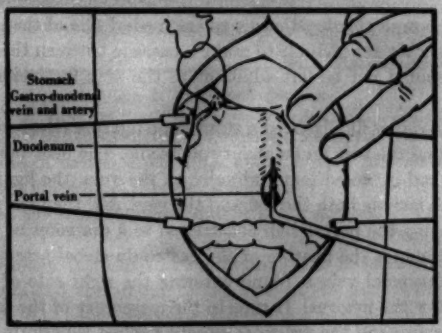

Fig. 1. The first operation.

The position of the anæsthetized animal is all important. A pad of cotton-wool 2 in. thick is placed under the animal's spine at the level of the last rib and the animal's four limbs tied down. The animal is in the

correct position when it is stretched over the pad so that the highest point of the abdominal wall is half-way between the umbilicus and the xyphisternum.

The abdomen is opened by an incision, 1 cm. to the left of the midline, from the costal margin to the level of the umbilicus. The reason for making the incision in the left side of the abdomen, at such a distance from the portal vein, is to minimize the danger of adhesions forming on the right side where the field of the second operation lies. The edges of the incision are retracted more strongly to the right than to the left so as to bring the incision over the portal vein. If the position of the animal is correct the upper part of the wound is occupied by the stomach, the lower by coils of intestine; the liver should not be visible.

The first step is the constriction of the portal vein. The great omentum is gently tucked up over the stomach and the portal vein identified below the pylorus in the angle between the duodenum and the lower border of the stomach. The tissue anterior to the vein is split longitudinally by fine forceps and the vein inspected for a transverse white line which indicates the correct place to form the constriction. When this is identified the portal vein is dissected free in its bed of tissue, the greatest care being taken not to open the peritoneum on the right side of the abdomen. This delicate operation is best performed by using small pledgelets of cotton-wool on the end of fine forceps for freeing the anterior and lateral aspects and a bent glass spatula to free the posterior aspect. Occasionally trouble is caused by lymph glands adhering to the medial side of the portal vein, but it is nearly always possible to make a passage between them next the vein. They should not be left adherent to the vein for inclusion in the ligature.

When the vein is finally freed a stout linen ligature (Barbour's No. 18) is carried round the vein on an aneurysm needle. The bent portion of the 2 mm. glass rod is placed longitudinally on the vein, the ligature is tied down so as to include both the rod and the vein, and then the glass rod is removed leaving the portal vein constricted to a diameter of 2 mm.

The next step is the ligation of the gastro-duodenal artery and vein. The gastro-duodenal vein is found entering the right side of the portal vein just below the pylorus. It runs in the mesentery of the first portion of the duodenum and the artery is closely applied superiorly. The peritoneum is punctured on either side of the vessels as near to the portal vein as possible. By means of bent forceps ligatures are carried round the vessels, tied, and the vessels divided between these ligatures. The small hole in the mesentery is closed by tying the free ends of the ligatures together.

100 c.c. of normal saline at 37° C. are poured into the abdominal cavity and the abdominal wall closed by continuous thread sutures in three layers—peritoneum, muscles, and skin.

Post-operative treatment. Apart from warmth until the animal has recovered from the anæsthetic no special measures are required.

Second operation (Fig. 2)

Three weeks should be allowed to elapse between the first and second operations. The preparation with regard to food, the anæsthesia and general technique are the same as for the first operation.

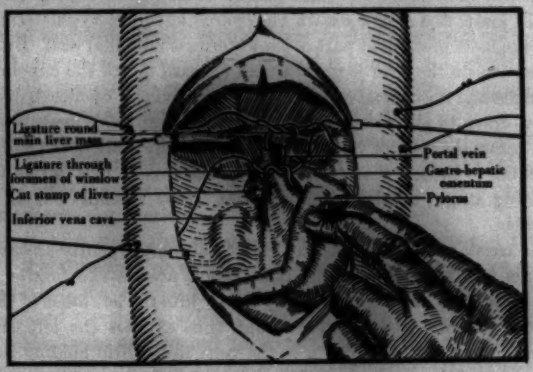

Fig. 2. The second operation.

Two special ligatures should be prepared. These consist of a soft centre portion made of cotton lamp-wick, about 3 mm. in diameter, knotted at each end to stout linen threads (Barbour's No. 18). The length between the knots of the centre portion should be in one case 12 cm. and in the other 9 cm.

The abdomen is opened in the midline, the incision starting on the xyphisternum and continuing to the level of the umbilicus. The xyphisternum is split to allow more effective retraction of the lips of the incision.

The stomach is gently drawn down a little way, the lesser omentum is punctured just above the pylorus, close to the portal vein and an aneurysm needle passed through the hole down and out of the foramen of Winslow. The point of the needle should emerge below the renal lobe of the liver ventral to the inferior vena cava. The special ligature, 9 cm. long, is

threaded into the needle, carried back and tied in a very loose half hitch so as to include the anterior wall of the foramen of Winslow. This ligature thus embraces the portal vein, the hepatic artery and the bile duct.

The stomach and intestines are drawn to the left and the renal lobe of the liver exposed. The ligament connecting the inferior pole of this lobe to the inferior vena cava is divided, a stout ligature passed round the pedicle of the lobe, and tied tightly. The ligated renal lobe is then cut off as close to the ligature as possible and the stump cauterized with pure carbolic acid.

The pad of cotton-wool which has up to now been resting under the animal's lower ribs is now drawn headwards until the xyphisternum becomes the most prominent part of the anterior abdominal wall. By means of a bone spoon the liver is made to fall forward out of the cupola of the diaphragm and the central coronary ligament divided, by means of curved scissors, down to the vena cava. The second lamp-wick ligature is then placed across the inferior vena cava so that slightly more of the lamp-wick portion lies to the left than to the right of the vessel. It is in this and the succeeding stages of the operation that Tilley forceps will be found invaluable.

The assistant now retracts the left costal margin gently upwards and outwards and by means of the bone spoon presses the fundus of the stomach caudally. The operator very gently lifts the left lobes of the liver from the posterior abdominal wall and thus exposes the left coronary ligament which is divided down to the vena cava. Still holding the liver the operator picks up the end of the lamp-wick ligature on that side and draws it under the left lobe of the liver to the front, bringing it up between the main mass of the liver and the small omental lobe of liver which lies in the folds of the lesser omentum against the upper surface of the stomach. The right end of the lamp-wick ligature is then brought to the front under the right lobe of the liver, and the two ends of lamp wick tied in a very loose half hitch. This ligature embraces all the vessels leading to and going from the main mass of liver.

Straight surgical needles are threaded on to the linen threads which are knotted to each end of the lamp-wick ligatures and these are passed through the lateral abdominal wall close to the costal margin. Care is taken to leave the threads quite slack within the abdomen. A few knots are tied in the threads coming from the ligature embracing the anterior wall of the foramen of Winslow to distinguish this ligature from the other.

The blood vessels of the great omentum are now ligated and the omentum completely removed. This is necessary to obviate the formation of adhesions to the liver. The removed omentum is cut into strips and these strips are wound round the ligatures within the abdomen. The rough ligatures themselves are apt to chafe or even erode the liver; when covered with omentum this does not happen. 100 c.c. of 10 p.c. glucose in 0·9 p.c. saline are poured into the abdominal cavity and the abdomen is closed in two layers—muscle and peritoneum, and skin. The linen ligatures protruding from the lateral abdominal wall are tied behind the rabbit's back and protected by bandaging the trunk of the animal.

Post-operative care. The animals are placed in a warm room and kept there until required. Every evening they receive subcutaneously 40 c.c. of 20 p.c. glucose in water. Ordinary food is allowed and usually at the end of 48 hr. the animals are eating quite well. In addition they are given 100 c.c. of 20 p.c. sucrose solution to drink each day.

The optimum time for carrying out the final step of excluding the liver is 4 days after the second operation. In this time the animal has recovered from the operation and any adhesions which have formed have not yet become vascularized.

Criteria of a successful preparation. A clinical impression that the state of the animal, 4 days after operation, is good is not an efficient criterion that the animal is physiologically normal. This point was demonstrated clearly on animals prepared by this method for experiments which were carried out to investigate the site of action of the glycotropic factor of the anterior pituitary gland [Himsworth & Scott]. Animals prepared as above, but in whom the ligatures were not tightened, were used as controls. If they were in perfect condition their response should have been positive and indistinguishable from that of unoperated animals. Animals which appeared clinically ill gave no response to the extract, but further, some which looked well also failed to respond. The following criteria of what should be regarded as a satisfactory preparation were ultimately arrived at on the ground that such animals responded normally to the pituitary extract.

The animal must appear to be in good clinical condition. It must neither have, nor have had diarrhœa.

The blood-sugar value before exclusion of the liver must be at least 100 mg./100 c.c. (Hagedorn-Jensen method).

As a further check at post-mortem the intestines must not be congested and the spleen must be of normal size. The liver must not be tense

and friable. The ratio of weight of liver to body weight should not be more than 0·056; higher values indicate the presence of vascularized adhesions.

Exclusion of the liver

To perform the exclusion the thread ligatures are gently freed where they pass through the abdominal wall, and each pair of threads is firmly and steadily drawn tight. The linen threads, which remain outside the abdominal wall, are then cut off and the stumps allowed to slide back into the abdomen. Once the knots inside the abdomen have been tightened no fear need be entertained of their slipping as the wet lamp-wick "jams" securely. The security with which such ligatures occlude the blood vessels in their grasp has been proved on many occasions. Rabbits have been anaesthetized at varying times after exclusion of the liver, the liver cut out above the ligatures, and never has bleeding occurred from the cut stumps of the liver vessels.

When the ligatures have been pulled tight the afferent blood supply to the whole liver is cut off and the efferent vessels from the main liver mass which constitutes 90–94 p.c. of the animal's liver, are also occluded. Thus 90–94 p.c. of the liver is completely shut out of the circulation, whilst the remaining 10–6 p.c. lies in a circulatory cul-de-sac, of which only the efferent vessels are open.

It will be remembered that in the second operation the renal lobe of the liver was removed. This lobe varies considerably in size in different animals, but the portion removed usually constitutes about 15 p.c. of the original amount of liver in the body. Control experiments have shown that the removal of this portion of liver has no effect upon the carbohydrate metabolism of the animals.

The spontaneous decline of the blood glucose after exclusion of the liver

Animals prepared by the above technique have been used for studying various aspects of carbohydrate metabolism; but for the purposes of comparing these preparations with those produced by the orthodox methods of hepatectomy figures showing the spontaneous decline of the blood sugar only will be considered.

Methods

Preparation. Food was withdrawn at 6 p.m. the previous evening. The animals were thoroughly warmed and throughout the experiment were kept in a warm box. Exclusion of the liver was performed in each case at 12 noon.

Animals. Seven male rabbits were used. The weight averaged 2·029 kg. (S.D. ∓0·0223). All satisfied the criteria which had been adopted to distinguish reliable preparations. The ratio liver weight/body weight averaged 0·0449 (S.D. ∓0·0109).

Blood sugar. The blood sugar was estimated in 0·1 c.c. of whole blood by the Hagedorn-Jensen method. Blood was drawn direct from a puncture in the ear vein. Before exclusion of the liver three samples were taken, and immediately after death two samples from the heart blood which was withdrawn by means of a needle and syringe. The first and last samples of the experiment, after deproteinization, were fermented with washed yeast and then the residual unfermentable reducing substance estimated [Somogyi, 1927]. The average of these two determinations was subtracted from the values given by the Hagedorn-Jensen method for the other samples to obtain the true blood glucose values of each sample. This procedure appears permissible as the average non-fermentable reducing substance in blood as estimated by this method in sixty-three separate samples from thirty-five different rabbits was 14·63 mg./100 c.c. (S.D. ±5·04). When fermentation has been employed the results will be designated blood glucose; when fermentation has been omitted the results will be called blood sugar.

It has been known for some time [Hemmingsen & Marks, 1932; Himsworth, 1934] that the higher the initial level of blood sugar the greater the absolute fall at definite times after the injection of insulin. The same rule appears to hold for the spontaneous decline of the blood glucose after exclusion of the liver. In 1932 Scott & Dotti showed that after injection of insulin into a series of rabbits uniformity in the insulin depression curves could be demonstrated if the blood sugar values were expressed not in absolute amounts but as percentages of the initial blood-sugar level. This method of expression has been adopted in this paper although, the initial blood glucose levels in this series of experiments being all close to each other, comparatively little difference in the figures has resulted.

Results

Clinical course. After occlusion of the liver the animals were usually restive for 5–10 min. They then became quiet and remained so for the rest of the experiment. The pulse rate rose and, judging from the ease with which blood specimens were obtained, the blood pressure did not fall. The respirations did not alter at first but later in the experiment they became deeper and slower, and when hypoglycæmic convulsions were imminent or had occurred Cheyne-Stokes breathing appeared. About

half an hour before convulsions occurred the animals became ataxic and later marked weakness was apparent so that the animal, when placed on its legs, sank down with sprawling limbs until the head rested on the floor. Just before a convulsion the animal often rolled on to its side. Twitching of muscles rarely preceded the convulsion, and the convulsions when they occurred were sudden and severe. Three or four severe convulsions at intervals of 5 or 10 min. take place and death occurs in a final convulsion which is usually distinguished by marked opisthotonus. On another series of animals the efficiency of parenteral glucose in restoring a convulsing animal to an apparently normal condition was demonstrated.

The average time after occlusion of the liver at which the first convulsion occurred was 278·5 min. (S.D. ±68·69). The average time of death was 291·8 min. (S.D. ±70·04).

Blood-glucose values. The average initial blood-glucose value was 100·1 mg./100 c.c. (S.D. ∓7·00).

The average terminal blood-glucose value, taken immediately after death in convulsions, was 19·5 mg./100 c.c. (S.D. ±3·86).

The decline of the blood glucose after exclusion of the liver. The average fall of blood glucose up to the average time of death was 81·5 mg./100 c.c. This fall is not uniform (Fig. 3). In the first 20 min. the blood glucose drops to 75 p.c. of the original value whilst it takes a further 100 min. to fall to 50 p.c. and a further 180 min. to reach 25 p.c. The curve is approximately hyperbolic. The smallness of the standard deviation of each blood glucose value, when expressed as a percentage, reveals a remarkable uniformity of decline in the different experiments. In no case was the fall smooth. This can be seen from the average curve (Fig. 3) but more clearly from the curve of one experiment (Fig. 4). It will be seen that the decline is interrupted by a series of waves. I am convinced that these waves are not artefacts and further work is in progress to test the possibility that there exists in the peripheral tissues a source of blood glucose.

Factors which affect the decline of the blood sugar after hepatectomy. When 90–94 p.c. of the liver is removed from rabbits under general anæsthesia by the method of McMasters & Drury [1929] the anæsthetic itself provokes an initial hyperglycæmia with the result that the blood-sugar level after hepatectomy is abnormally high, and the glycogen reserves which have supplied the blood sugar are correspondingly depleted [Murphy & Young, 1932]. In my hands hepatectomy by McMasters & Drury's method resulted in a fall of blood sugar at the uniform rate of approximately 11 mg./100 c.c./hr., although Drury & Salter [1934] have obtained uniform falls of 20–25 mg./100 c.c./hr. The figure I obtained

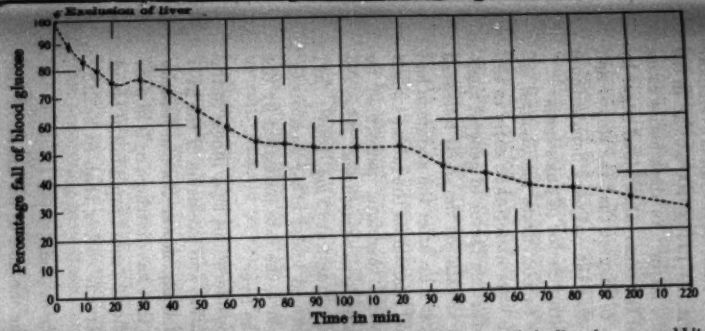

Fig. 3. Curve showing the average spontaneous fall of blood glucose after exclusion of the liver in seven rabbits. The liver was excluded at 0 min. The points through which the curve is drawn represent the mean values for the seven rabbits; the vertical lines drawn from these points show the standard deviation of these values.

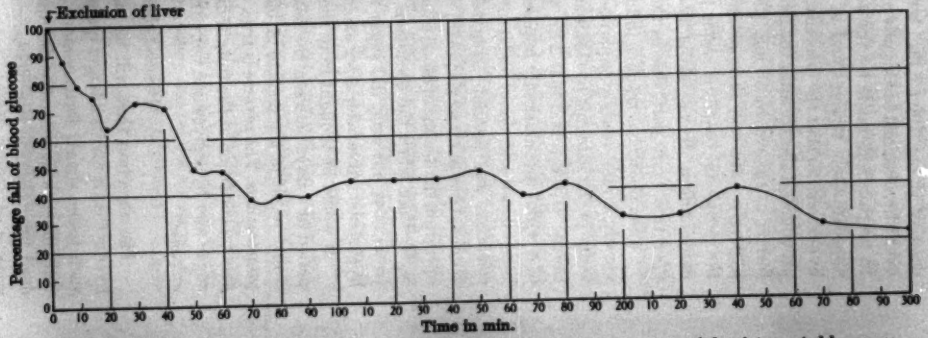

Fig. 4. Spontaneous fall of the blood glucose in a rabbit, showing that the fall is not smooth but interrupted by waves.

agrees well with the average fall obtained by Mann & Magath [1922], in dogs, of 12 mg./100 c.c./hr., but is slower than even the average decline over 5 hr. obtained by this new method of exclusion which is 16 mg./100 c.c. In preparations made by the acute operation the curve of the decline is approximately straight and in those made by the method of exclusion hyperbolic, so that the discrepancy in rate of blood sugar fall is even more pronounced shortly after the liver is cut out; 10–15 mg./100 c.c./hr. in the former as against 40–50 mg./100 c.c. in the latter. As anæsthetics cause hyperglycæmia and also prevent or retard the removal of blood sugar by the peripheral tissues it seems justifiable to suggest that the slower and more uniform decline of the blood sugar after the ordinary hepatectomy operation, is, in part, due to the complicating factor of anæsthesia.

That operative trauma influences the carbohydrate metabolism adversely is indicated by experiments which were undertaken early in this series in the attempt to eliminate the disturbing factor of general anæsthesia. It was found that 2 hr. after ether anæsthesia the sugar tolerance of a rabbit was markedly impaired, but that 2 hr. after the abdomen had been opened and closed under local or spinal anæsthesia the rabbit's sugar tolerance was normal. Hepatectomies were, therefore, performed by McMasters & Drury's [1929] method using local anæsthesia. After the operation the decline of blood sugar was uniform and, at 13 mg./100 c.c./hr., faster than when the operation was performed under ether anæsthesia. In no case, however, did sugar removal reach the rate indicated by the hyperbolic curve seen after exclusion of the liver.

It would be thought from the above considerations that illness in the period of 4 days between the second operation and exclusion of the liver would result in the decline of blood sugar being slower than in healthy preparations. The reverse is true. A rabbit that has diarrhœa or is in poor general condition at the time of exclusion shows a more rapid fall of blood sugar and hypoglycæmic convulsions supervene earlier than in healthy animals.

Possible objection to the method

The objection that will be raised to the method of exclusion of the liver is that it leaves a larger quantity of dead liver tissue in situ and that such tissue should form noxious products which, acting on the animal, will influence significantly the course of events. This objection to leaving dead liver tissue in situ has been stressed by Mann [1927] and Markowitz *et al.* [1933]. In support of this contention the work of Ellis & Dragstedt [1930] has been misquoted. These authors did not show that liver tissue autolysing in vivo was toxic, on the contrary they showed it

was innocuous. What they did show was that the livers of adult dogs are infected with a gas-forming anærobe which acts on dead liver tissue to produce highly toxic products and that it is to these that fatal poisoning is to be attributed. In such cases the portions of dead liver are found as grey necrotic masses full of gas bubbles. In no case have I seen gas formation in rabbits' livers which have been excluded from circulation in the rabbit's abdomen. Conclusive evidence that dead liver tissue remaining in situ is not highly toxic to the rabbit is provided by the work of Cameron & Mayes [1930] on ligation of the hepatic artery. In these experiments the hepatic artery was tied with consequent infarction of portions of the liver, but the portal vein and hepatic veins were left untouched. Any toxic products formed in the necrotic infarcts could thus reach the general circulation quickly. The authors report that the animals may die within 10 days or not uncommonly live as long as 84 days without showing any obvious signs of ill-health apart from a decided loss of weight. This is in marked contrast to the results on dogs [Ellis & Dragstedt, 1930; Mason & Davidson, 1925] in which 100 g. of dead liver tissue in the abdomen produced death in 15–60 hr.

In the method of exclusion of the liver described in this paper the major portion of the liver is completely cut off from the circulation. A small portion which weighs between 5 and 9 g. has its afferent vessels occluded but remains in communication with the inferior vena cava through its hepatic vein. This portion is a great deal smaller than the necrotic infarcts produced in Cameron & Mayes's experiments, and further, any toxic products of autolysis formed in it are not washed into the circulation by blood circulating through the liver but can only reach the blood stream by diffusing through the hepatic veins into the inferior vena cava.

From these considerations I cannot think that, in the relatively short survival period of animals whose liver has been excluded, toxic products from autolysing liver tissue materially influence the results obtained. In this opinion I am supported by the constancy and ease of reduplication of results on these preparations.

Summary

A method is described for the instantaneous and painless exclusion of the liver from the circulation in the intact, unanæsthetized rabbit.

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BINOCULAR SUMMATION DURING DARK ADAPTATION

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In spite of the considerable amount of work which has been done on the problem, it is still doubtful whether the smallest illumination visible during adaptation to darkness is less when observations are made binocularly than when they are made monocularly. Many authors deny altogether that there is any binocular summation as measured by the light threshold [Charpentier, 1888; Wölfflin, 1905; Lasareff, 1914; Downey, 1919; Müller, 1922; and others]. Piper [1903*a*] thought that summation occurred only after 15 min. adaptation to darkness and that the binocular was then roughly half the monocular threshold. Other workers found similar results. Roelofs & Zeeman [1914] found summation in light adaptation, and throughout dark adaptation. Their paper contains a good résumé of work in this field previous to 1914. The only quantitative work which carries conviction is that of Cook [1934]. He found binocular summation, but his results were confined to a small test patch observed foveally. Cook's paper contains a critical survey of previous work.

The object of the present experiments was to obtain quantitative measurements of the monocular and binocular thresholds during adaptation to darkness for a large test patch using a composite white light.

Method

In the present experiments we obtained first, a complete run with both eyes, secondly, a complete run with the right eye, and finally, one with the left eye. The three runs with the intermediate periods of adaptation to a bright light were completed at one sitting. Subsequently the order was changed until, after six sittings, the results were averaged and compared.

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Examination of published results shows that the values of the light threshold, when plotted against the time in the dark, rarely fall on smooth curves as they should. This is particularly true when binocular and monocular thresholds are measured alternately during the same period of dark adaptation. Changing the conditions of the experiment during a run renders less reliable a form of observation which by its nature is already very unreliable. We believe that smooth repeatable curves can be obtained only by a strict attention to the experimental details, and that more reliable results are obtained when whole runs are compared than when the conditions are changed in the middle of a run.

After the preliminary period of light adaptation the observer was plunged into darkness, and a predetermined illumination was projected on the test patch for 0·2 sec. every 2·5 sec. The time taken for the observer to see a flash was recorded. The illumination was then immediately reduced, and as before, the time taken to see the new illumination was recorded. This operation was repeated for 20 min. The use of the same predetermined illuminations for every run makes it possible to find the average time at which the same illumination was seen during different sittings under the same conditions. The method is not satisfactory for longer periods of dark adaptation since, after about 20 min., the light threshold decreases too slowly.

The observer was instructed to signal to the experimenter only when he was quite sure that he could see the flash whilst maintaining accurate fixation. This method assumes that the observer neither signalled before the illumination was visible nor allowed too long a time to elapse before pressing the key. More elaborate psycho-physical methods in which a check is kept on the observer, were not possible on account of the very rapid change of sensitivity during early dark adaptation. In addition the observers were not able to maintain accurate fixation for every flash, and any deviation of the eyes was liable to cause a change in the threshold.

Even in the complete absence of light the visual world does not look black but grey (self light of the retina). It is difficult for an observer to be sure that the sensation of light comes from the test patch and is not due to this self light. A flashing light has been found to make observation considerably easier.

Prolonged fixation of an illuminated test patch may lower the sensitivity of the eye even though the illumination is subliminal. Even with discontinuous light the sensitivity of the eye at any given time of dark adaptation may be less than it would have been if light had been completely excluded from the eye up to that time. If the sensitivity of one

eye is lowered in this way it follows that one cannot begin to take observations with the previously covered eye half-way through an experiment and hope to obtain an accurate comparison of the thresholds of the two eyes.

It was, however, impossible to demonstrate any alteration in the level of dark adaptation produced by the test patch itself because the effect, if any, was completely swamped by another phenomenon. In preliminary work it was found that any interruption in a set of readings immediately produced very erratic results which could not be reduced to any sort of order. Travis [1926] has shown that at the threshold the eye exhibits a phenomenon akin to that of the refractory period in muscle and nerve, and this phenomenon may in part explain the erratic readings. Whatever the cause, the practical outcome is that it is necessary to make a complete run under one set of conditions and to compare it with other sets under different conditions. Any errors introduced by this method are the same for all experiments. Progressive changes in sensitivity such as the onset of fatigue may occur during the three runs which comprise one sitting. This source of error can be eliminated by taking the three runs in every possible order and averaging the results. Although this precaution was taken it was found that progressive changes from one run to another, if present, must be quite small. This was shown by averaging all runs taken first, all runs taken second, and all runs taken third in a sitting by each observer. The resulting average figures were almost identical, showing that there had been no progressive and consistent change during a set of three runs taken at one sitting.

The pupil diameter is known to fluctuate considerably in the dark [Lythgoe, 1932; Crawford, 1936], and it is also probable that the diameter is not the same for binocular as for monocular observation of the test patch [Schubert & Burian, 1936]. Since the design of artificial pupils suitable for binocular observation in the dark presents many difficulties, it was decided to use a mydriatic. The latter was made of β-euphthalmine 2 p.c., cocaine hydrochloride 2 p.c. in 0·4 p.c. NaCl solution. When used as described below the drops produced a lasting maximal dilatation of the pupil with very little paralysis of accommodation. Infra-red photographs showed that the diameter of the pupil under these conditions was the same both for monocular and binocular observation.

Apparatus

The general form of the apparatus is shown in Fig. 1. The subject sat in a white octagonal chamber facing the wall *A*. During light adaptation the entire chamber was uniformly illuminated by banks of 100 W. lamps shining into the chamber through ground-glass screens. The maximum brightness of the walls as measured by a lumeter was 130 equivalent foot-candles.

The illumination of the test patch was produced by projecting an image of one side of the opal glass screen G on the wall A by means of the lens C. The opal glass was illuminated by motor head-lamp bulbs mounted on its far side. In order to produce a wide range of illuminations at the test patch two lamps (12 V. 60 W. and 12 V. 12 W.) were mounted on heavy steel bases which could be moved smoothly along two $\frac{1}{4}$ in. steel rods B forming an optical bench 3 m. long, giving a 100 : 1 variation in the illumination of the test patch for any one lamp. Two diaphragms were used in the lens and these in combination with the two lamps gave an over-all variation of 25,000 : 1. The lamps were adequately screened.

The test patch illuminations were measured directly by a substitution method. For this an additional 500 W. lamp was necessary and the candle-powers of this and the 60 and 12 W. lamps were then calibrated against a similar lamp which had been standardized by the National Physical Laboratory. For this purpose a Weston photronic cell was placed at G (Fig. 1), and the positions on the optical bench determined at which each lamp gave the same deflexion of the galvanometer in series with the photocell. The candle-powers were

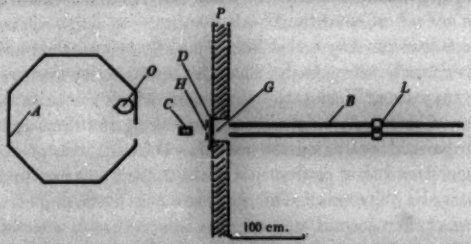

Fig. 1. Plan of apparatus. O, observer; A, test patch; C, lens; H, aperture; D, shutter; P, partition; G, opal screen; B, optical bench; L, lamp.

then calculated using the inverse square law. The photocell was then placed at the centre of the test patch (A) with the 500 W. lamp in one of the standard positions on the optical bench. The deflexion of the galvanometer was noted. The optical parts of the system (the lens and the opal glass screen) were then removed and the 12 W. lamp was placed at such a distance on the optical bench that the same deflexion was obtained. The illumination of the test patch by the 12 W. lamp was calculated using the inverse square law. An identical illumination was produced by the 500 W. lamp at one position on the optical bench with the lens and opal glass in position. The illuminations produced by other lamps in other positions could be calculated readily. The advantages of this method of calibration are that the illumination is measured at the test patch itself; that it does not depend on the linearity of the photocell; and that it involves no assumptions as to the passage of light through the optical parts of the apparatus. The brightnesses of the test patch in equivalent foot-candles were obtained by multiplying the illumination of the test patch by its reflexion factor. The range so obtained was from $5{\cdot}6 \times 10^{-8}$ to $2{\cdot}2 \times 10^{-7}$ equiv. f.c.

Although the lamps were underrun at an average voltage of 10·9 they nevertheless showed some ageing. They were calibrated frequently and their candle-powers were kept constant by voltage adjustments.

The visual angle subtended by the test patch was $12\frac{1}{2}°$. The flashes were produced by a rotating contact and a quick-acting electromagnetic shutter (D). A flash occurred every 2·5 sec. and lasted 0·2 sec. A small red spot of light (Wratten No. 25) projected at a spot corresponding to the centre of the test patch, served as a fixation light. Its brightness could

be controlled by the observer. Owing to the large size of the test patch, observations were really made with circumfoveal vision, although the test patch was fixated centrally. The observer's head was kept steady by comfortable supports.

On account of the rapid increase in sensitivity during the early stages of dark adaptation, the beginning of dark adaptation, the responses of the observer, the time of each flash and 5 sec. time signals were all recorded by a 3-pen marker on a moving band of paper.

There were five observers:

G. F. K., æt. 27, a schoolmaster, who although untrained as an observer, showed special aptitude for this type of experiment. Emmetrope, with no ophthalmological defect.

R. J. L., æt. 40, an experienced observer. Slight myopia, about −0·5 D.Sph. 5° exophoria for near vision.

A. M., æt. 27, a Ph.D. student with no previous training in subjective observations. He experienced difficulty in observing, which he ascribed to apparent movements in the visual field in the dark. Emmetropic.

L. R. P., æt. 32, a trained observer in this type of experiment. Emmetropic.

E. S., æt. 26, a research student with no previous training in subjective observations. A myope, −1·75 D.Sph. in each eye. No other abnormality. He did not wear his glasses for the experiments and had great difficulty in observing.

Experimental procedure

The observer received 1 drop of the mydriatic in each eye every ½ hr. for 1 hr. before the initial period of light adaptation. He then entered the chamber and was light adapted for 9 min.; 2 min. at 28 equiv. f.c., 2 min. at 130 equiv. f.c. and 5 min. at 28 equiv. f.c. The lights were then abruptly switched off and the highest available illumination was projected on the test patch. Using both eyes, the observer signalled immediately he saw the first flash. The test-patch illumination was then reduced and the observer again signalled when he saw the flash. Readings were continued in this way for 20 min. After receiving a further drop of mydriatic in each eye, light adaptation was repeated for a second period of 9 min. and another dark-adaptation run was made with the right eye. A third period of light adaptation and a run with the left eye completed the sitting. Five further sittings were made, observations with both eyes, the right eye and the left being made in all possible combinations. The readings for both eyes, and for each eye separately, were then averaged. It should be noted that in every case light adaptation was binocular and uniform for both eyes. Switching off the light was the signal to cover the non-observing eye with a shade when a monocular run was about to be made.

Results

Four of the five observers completed a set of readings with central fixation. Two observers (R. J. L. and L. R. P.) did a set of readings for the peripheral retina in which the fixation spot was 25° distant from the

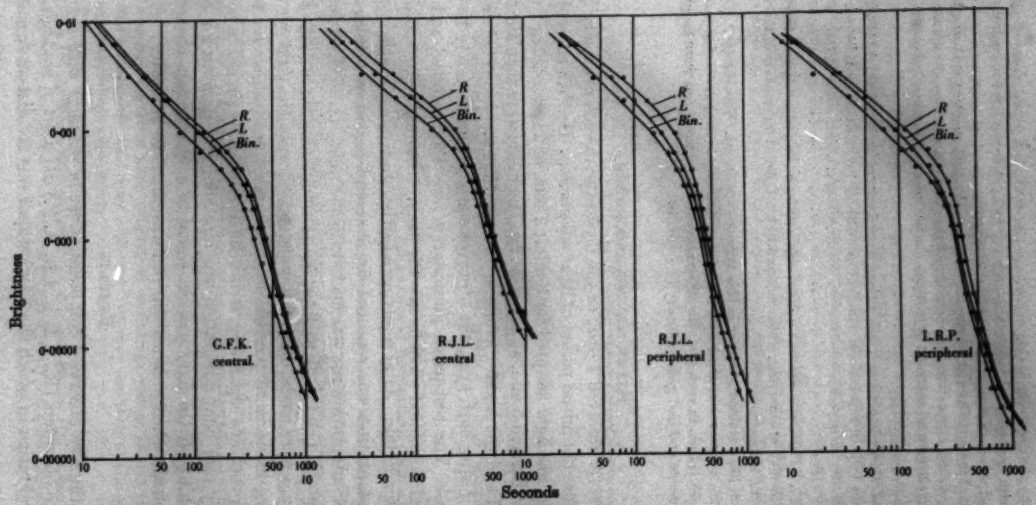

Fig. 2. Binocular (*Bin.*) and monocular (*R* and *L*) thresholds for central and peripheral vision. Ordinate: brightnesses of the test patch in equivalent foot-candles (log. scale). Abscissae: duration of dark adaptation in seconds (log. scale). The initials of the observers are printed against the curves. (L. R. P.'s peripheral curves appear to cross, but do not, in fact, do so.)

TABLE I. Observer, R. J. L. Central vision. Observed times in seconds for individual runs at which various brightnesses were seen. $B = 10 + \log$. (brightness of test patch in equivalent foot-candles). The numbers in *italics* at the heads of the columns give the chronological order in which the runs were taken.

	Binocular							Right eye							Left eye						
B	*1*	*5*	*9*	*11*	*15*	*16*	Mean	*2*	*4*	*7*	*12*	*14*	*18*	Mean	*3*	*6*	*8*	*10*	*13*	*17*	Mean
7·75	16	17	19	16	23	16	18	20	20	20	27	27	25	23	28	27	25	30	26	25	27
7·45	29	27	35	30	44	30	33	34	27	40	54	64	45	44	60	72	53	71	63	68	65
7·23	53	48	81	78	89	57	68	84	55	85	118	97	108	91	133	128	107	109	114	111	115
6·93	113	114	174	147	177	144	145	217	101	227	230	197	170	190	237	235	192	286	227	233	235
6·75	258	191	222	215	234	234	226	274	234	294	296	262	245	268	289	277	251	325	328	290	293
6·59	312	237	271	262	267	282	272	324	278	322	345	294	307	312	350	325	282	390	388	313	341
6·45	344	270	298	292	324	351	313	357	312	371	377	338	350	351	386	343	336	422	407	356	375
6·35	385	311	336	318	348	355	342	377	363	410	396	367	388	384	413	379	360	457	439	390	406
6·23	430	331	363	353	363	410	375	415	385	440	423	405	407	413	438	412	400	479	486	410	438
6·05	470	373	417	394	410	474	423	468	455	484	470	440	442	460	488	451	450	549	541	481	493
5·95	520	424	452	423	454	514	465	489	495	504	486	463	487	487	507	503	473	579	588	502	525
5·72	555	466	479	467	487	600	516	555	535	568	527	528	560	545	575	567	548	650	667	540	591
5·42	667	560	592	570	628	690	618	700	706	644	686	687	878	717	668	668	725	820	955	750	764
5·25	764	620	732	648	743	885	732	833	943	724	777	971	993	874	870	907	919	912	1062	1073	957
5·09	925	717	824	735	938	1230	895	1093	1020	852	1023	1026	1138	1025	1115	964	1070	1070	1204	1153	1096

TABLE II. Observed and calculated binocular thresholds for four observers using central, and for two observers using peripheral vision. Observed thresholds (Th_R, Th_L and Th_B obs.) were read directly from graphs similar to those shown in Fig. 2. Calculated binocular thresholds (Th_B calc.) were obtained by substitution of the observed monocular thresholds (Th_R and Th_L), in equation (1). For convenience the results are quoted as $\log_{\cdot 10}$(threshold brightness) + 10. Brightnesses are in equivalent foot-candles.

	R. J. L. central				R. J. L. peripheral				L. R. P. peripheral				G. F. K. central				A. M. central				E. S. central			
Time sec.	Th_R	Th_L	Th_B Obs.	Th_B Calc.	Th_R	Th_L	Th_B Obs.	Th_B Calc.	Th_R	Th_L	Th_B Obs.	Th_B Calc.	Th_R	Th_L	Th_B Obs.	Th_B Calc.	Th_R	Th_L	Th_B Obs.	Th_B Calc.	Th_R	Th_L	Th_B Obs.	Th_B Calc.
10	—	—	—	—	—	—	—	—	7·84	7·80	7·69	7·67	—	—	—	—	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	7·65	7·62	7·49	7·49	7·90	7·87	7·74	7·74	7·48	7·52	7·35	7·35	7·76	7·76	7·65	7·61
25	7·75	7·83	7·61	7·64	7·88	7·83	7·74	7·71	7·49	7·45	7·33	7·32	7·68	7·65	7·50	7·52	7·24	7·35	7·14	7·15	7·59	7·60	7·49	7·45
40	7·54	7·67	7·42	7·46	7·71	7·66	7·56	7·54	7·33	7·29	7·16	7·16	7·46	7·42	7·29	7·29	7·01	7·18	6·92	6·95	7·43	7·46	7·33	7·30
63	7·38	7·51	7·29	7·30	7·56	7·50	7·38	7·38	7·17	7·12	6·99	6·99	7·24	7·20	7·06	7·07	6·80	6·97	6·70	6·74	7·29	7·33	7·16	7·16
100	7·24	7·34	7·15	7·14	7·40	7·29	7·20	7·20	7·00	6·92	6·80	6·81	7·03	7·00	6·86	6·87	6·55	6·69	6·49	6·48	7·14	7·19	6·99	7·02
158	7·06	7·16	6·95	6·96	7·21	7·06	6·98	7·00	6·81	6·70	6·58	6·60	6·87	6·82	6·67	6·70	6·27	6·38	6·22	6·18	6·99	7·01	6·81	6·85
251	6·85	6·95	6·70	6·75	6·94	6·76	6·65	6·71	6·56	6·36	6·28	6·32	6·68	6·59	6·39	6·49	5·93	6·10	5·87	5·87	6·85	6·69	6·61	6·63
398	6·35	6·44	6·22	6·24	6·38	6·17	6·04	6·14	5·76	5·53	5·58	5·51	6·15	6·05	5·82	5·95	5·50	5·65	5·35	5·43	6·42	6·19	6·08	6·17
631	5·60	5·72	5·45	5·51	5·28	5·22	5·06	5·10	4·83	4·77	4·62	4·65	5·40	5·30	5·08	5·20	5·02	5·08	4·85	4·90	5·66	5·54	5·34	5·47
1000	5·20	5·26	5·07	5·08	4·64	4·64	4·48	4·49	4·35	4·30	4·19	4·18	4·76	4·68	4·52	4·57	4·55	4·62	4·41	4·44	5·06	5·03	4·88	4·90

centre of the test patch and a little higher. Graphs of the results for two observers (R. J. L. and G. F. K.), using central fixation, and two (R. J. L. and L. R. P.), using peripheral vision, are shown in Fig. 2. For convenience both the threshold brightnesses and the average times at which they were seen are plotted on a logarithmic scale. Each point is the mean of six observations taken at different sittings. It will be seen from the figures that the right and left eyes behaved differently for all observers. A typical set of results for one observer (R. J. L. central) is shown in Table I.

For all observers, and for all stages of dark adaptation, the binocular threshold is lower than either of the monocular thresholds. Under the conditions of these experiments it can be stated that binocular summation occurs at all stages of dark adaptation.

An analysis of the curves shows that the relation between the thresholds at any time is approximately expressed by the equation:

$$1{\cdot}4Th_B = \tfrac{1}{2}(Th_R + Th_L), \qquad \text{.....(1)}$$

where Th_B, Th_R and Th_L represent the light thresholds for binocular, right-eyed and left-eyed observations respectively.

The agreement of the various observers with this formula is shown in Table II. The figures quoted there were obtained from Fig. 2 (and from similar figures for observers A. M. and E. S.) by reading off the threshold brightnesses at various times during dark adaptation. The column which is marked "Th_B obs." gives the logarithms of the binocular threshold brightnesses (equiv. f.c. $\times 10^{10}$) at the times stated: the column which is marked "Th_B calc." gives the logarithms of the binocular thresholds calculated by formula 1 from the experimental values of the monocular thresholds. The observed and calculated values agree well for all observers and for all stages of dark adaptation with the possible exception of the period at about 7 min. The poorest agreement is shown by the myope E. S. who found observation difficult. This observer was uncorrected by glasses which he is accustomed to wear.

Discussion

It is a remarkable fact that the equation (1) holds for all experiments, for both the central and peripheral retinae and for all stages of dark adaptation. We have elaborated a theoretical explanation which seems to be reasonable but cannot be regarded as more than a suggestion until more experimental work has been done.

Piper [1903*b*] has shown that for a square test patch, whose diagonal subtends a visual angle not exceeding 14°, the light threshold (Th) is

inversely proportional to the diameter of the test patch. At the threshold, therefore, $Th\sqrt{\text{area}} = \text{constant}$. Löhle [1929] and Reeves [1918] found that the law was roughly true and the deviations from it are not sufficiently great to affect the argument which follows. If in our own experiments, the monocular threshold at any stage of dark adaptation were Th_M, we should, using Piper's law, expect the threshold for twice the area to be $Th_M \div \sqrt{2}$. Let us suppose that observation of the test patch with two eyes simultaneously is equivalent to looking at a patch of twice the area with one eye. If this is true, the binocular threshold should be identical with the monocular threshold for double the area, i.e.

$$Th_B = Th_M \div \sqrt{2} \quad \text{or} \quad 1{\cdot}4Th_B = Th_M. \qquad \ldots\ldots(2)$$

If both eyes were equal we could substitute the values for either eye in Th_M. With two unequal eyes the simplest assumption is that Th_M is the mean of the thresholds of the right and left eyes. By substituting $\frac{1}{2}(Th_R + Th_L)$ for Th_M in equation (2) we obtain the same equation as was derived empirically in equation (1). It appears, therefore, that the threshold for binocular vision is the same as that of an eye having the average properties of the two eyes viewing a test patch of twice the area.

Equation (1) would be obviously untrue if the observer had only one eye or Th_R were very large. Theoretically it is more desirable to work in terms of the sensitivities, S_B, S_R and S_L of both eyes, right and left eyes respectively, where sensitivity is measured by the reciprocal of the threshold brightness. The equation relating the three quantities becomes $S_B \div 1{\cdot}4 = \frac{1}{2}(S_R + S_L)$. This is the same as equation (1) when $S_R = S_L$ and $Th_R = Th_L$, and differs very little from it when Th_R and Th_L are nearly equal to one another.

Piper's law holds only for visual angles between 2 and 14°. For small areas viewed with central vision, the light threshold is inversely proportional to the area of the test patch (Riccò's law), and if the hypothesis outlined above is true we should expect the binocular threshold to be half the monocular threshold. Equation (1) would then become

$$2Th_B = \tfrac{1}{2}(Th_R + Th_L).$$

It has been shown by Löhle [1929] that Riccò's law does not hold except for very small areas subtending not more than 10 min. to the eye. There are no data for monocular and binocular thresholds for such small areas. Cook [1934] used a test patch subtending 55 min. to the eye. Riccò's law would not hold for anything so large, but one would expect the constant in equation (1) to lie between 1·4 and 2. Cook summarizes his results by saying that "the average monocular thresholds...were about 50 per cent higher than the binocular thresholds". An analysis of his results shows, however, that the relation between the binocular and monocular thresholds would be fairly well represented by equation (1),

except that the constant instead of being 1·4 is 1·6, 1·52, 1·54 and 1·76 in four cases taken at random. With even smaller areas, it is possible that the constant would approximate to 2.

Summary

1. Monocular and binocular light thresholds have been measured during the first 20 min. of dark adaptation for a white stimulus subtending 12½° at the eye. Central observation was used for four observers and peripheral observation was used for two observers.

2. Binocular summation was found throughout the 20 min. of dark adaptation for all subjects and for both central and peripheral vision.

3. The results can be expressed approximately by the equation

$$1{\cdot}4Th_{\text{Binoc.}} = \tfrac{1}{2}(Th_{\text{Right}} + Th_{\text{Left}}).$$

4. It is shown that the binocular threshold is approximately the same as the monocular threshold for a test patch of twice the area.

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THE ACTION OF CURARINE ON THE RESPIRATORY MECHANISM

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Curarine is the type alkaloid of Guianese curare: it has the classical curare action of neuromuscular paralysis. When given subcutaneously, the respiratory muscles are often among the last affected. But this is not always so, and in a number of species, notably in rodents and in dogs, animals whose general musculature is not yet noticeably weakened by the drug may be seized with respiratory embarrassment leading to cyanosis, and thence rapidly to fatal asphyxia [West, 1935].

The attacks of difficulty in breathing are as follows. The injected animal suddenly commences to breathe slowly and with effort, the difficulty appearing to be inspiratory rather than expiratory. At the same time a characteristic feature of curarization of the respiratory mechanism may develop, namely a long maintenance of the chest in the expiratory position, the inspiratory phase being represented by a short deep gasp sometimes divided into two stages, due to lack of synchronization of the intercostal muscles with the diaphragm, the former usually contracting first. Small animals (rats or guinea-pigs) will make pawing movements at their throats such as occurs with histamine, and respiration becomes audible as well as effortful in a way strongly suggesting bronchial spasm. In dogs, the independence of this respiratory attack from general curarization may be striking. The hitherto normal animal suddenly stands still, braces its forelegs, and breathes stertorously, slowly, and with inspiratory difficulty. The attack may remit after a few seconds, recur, and finally pass off, or else it may lead to a fatal asphyxia, in which case it is usually accompanied by some degree of general curarization. In two such cases it was found impossible to induce an air current in the trachea when artificial respiration was attempted by compression of the chest. Abductor paralysis of the vocal cords was considered as a cause of the

respiratory embarrassment, but was ruled out because tracheotomy did not remedy the condition [West, 1935]. Post-mortem examination of the lungs showed a remarkable degree of general collapse, the lungs appearing almost airless, though in fact the alveoli contained air and the lung floated on water. Curarized rabbits, killed before respiration is arrested, have collapsed, dark pink and almost airless lungs, in contrast to the partly distended, light pink, air-containing lungs of a normal control. An air-pressure of 8 cm. of water is required to distend the curarized rabbit lung, as opposed to a pressure of 2 cm. of water in death from a blow, or 3·5 cm. in death from ether anæsthesia.

Drug antagonism of curarine in its respiratory action

(1) Respiratory spasm in cats, produced by curarine, yields to adrenaline, and returns when the transitory effects of that drug have passed [West, 1937].

(2) Isolated rings of the trachea and large bronchi of the cat were examined in oxygenated Ringer-Locke solution. Curarine causes strong muscular contraction after a long latent period. This is only found with high concentrations of the drug (1 : 3000 and upwards). The spasm is unaffected by atropine (1 : 250,000), but removed by adrenaline (1 : 250,000) [West, 1937].

(3) With the collaboration of Dr J. W. Thornton, a few experiments were performed using his technique of perfusing the exposed lung of the guinea-pig and recording the perfusion-pressure as an index of bronchoconstriction. By this method also curarine produced measurable bronchoconstriction only when perfused through the lung at a concentration of 1 : 3000, in contrast with histamine which caused profound constriction at dilutions of 1 : 100,000.

The subject for investigation was twofold. First, in what ways can curarization, in its ordinary sense of myoneural paralysis, affect the mechanism of respiration? Secondly, to what extent has curarine an action on the respiratory mechanism other than by curarization?

Methods

Passive collapse of the lung was produced by phrenic nerve section and by curarization, and studied by X-ray and records of intrapleural pressures. The effects of anæsthetics were compared with those of curarine. The effects of posture on artificial respiration were also studied in this connexion.

The element of *bronchial spasm* was studied in the exposed lung of the guinea-pig by direct observation, and by records of distension pressures from a manometer attached to the tracheal tube. Curarine was compared with histamine by these methods.

Passive collapse of the lung

Diaphragmatic paralysis—phrenic nerve section. Complete motor paralysis of the rabbit's diaphragm was produced by bilateral section of the phrenic nerves in the neck. Ether anæsthesia was continued for 15 min., and the animal then killed by a blow on the neck. The lower lobes of both lungs were partially collapsed. In response to internal pressure, the upper lobes expanded as in the normal control, the lower lobes with greater difficulty. Diaphragmatic paralysis by motor nerve section does not produce the general collapse of the lung which occurs in curarine poisoning.

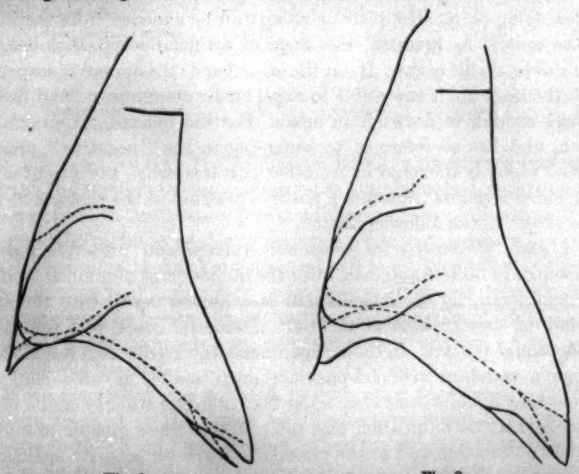

Fig. 1. Fig. 2.

Line drawings from a tracing of X-ray photographs. Decerebrate cat in horizontal position, lateral view. Dotted lines show reduction in size of thoracic cavity in curarization. Fig. (1), expiration. Fig. (2), inspiration.

X-ray observations: effect of posture. By the courtesy of the Director of the Nuffield Institute of Medical Research, Oxford, and the kind co-operation of the Honorary Radiologist, Dr A. E. Barclay, X-ray obser-

vations were made and X-ray cinematograph records taken, of rabbits and cats, under curarine, and in various postures. If a normal rabbit held with the head up is photographed, the chest appears elongated, and respiratory movements appear to be almost entirely diaphragmatic. If held inverted, the diaphragm advances a little into the chest cavity, the cavity widens laterally and the intercostal muscles take an increased part in respiratory activity. After curarization, the diaphragm rises into the chest by about one intercostal space with the head held up: its cephalic displacement being more with the head down. Further, with the head up, the chest cavity becomes diminished in all its measurements: inverted there is no lateral expansion adequate to compensate for the altered position of the paralysed diaphragm. The volume of the thoracic cavity must be markedly reduced by such curarization (Figs. 1 and 2).

Posture and artificial respiration by compression. If the chest wall of the curarized rabbit or curarized decerebrate cat be compressed with the animal lying on its side, little or no air can be expelled from the lungs. If the animal be inverted, this form of artificial respiration becomes impossible; no air passes. If, on the other hand, the animal is suspended with the head up, it is possible to expel air by compression, and there is enough suction to draw air in again. For the relaxed diaphragm has fallen, and has so reformed to some degree the "negative" pressure system which is abolished in the other postures, when the weight of the abdominal contents transmits a positive pressure to the thoracic cavity, as is shown in the following section.

A study of intrapleural pressures. Intrapleural pressures may be registered, in rabbits and cats, after the induction of a minimal artificial pneumothorax, by the introduction of a hollow needle into the space behind the sternum at level of the 4th intercostal space, with the animal in the supine posture. In these experiments the needle used resembled in design a standard artificial-pneumothorax needle, as used clinically, except that it had a somewhat wider bore, a firmer stillette, and a longer stem beyond its bifurcation and tap. It thus gave greater protection against obstruction and at the same time a greater security against the adventitious introduction of air through the main stem. Before its insertion the needle was connected with a water manometer by a narrow-bore pressure-tube. The needle was introduced with a "negative" pressure of about 1 cm. of water already in the closed system, so that very little air entered the pleural cavity. Free oscillations were obtainable without difficulty. In the cat, under moderate ether or chloralose anæsthesia, pressures in expiration varied from $-0{\cdot}5$ to $-1{\cdot}5$ cm. water,

in inspiration from −1·5 to −2·5 cm. In the rabbit, under ether alone, the pressures were more variable. In expiration there was seldom less than −1 cm. on induction, and, under light anæsthesia, inspiratory pressures reached −4·5 cm. Average established pressures for the rabbit were: expiration −0·5 cm., inspiration −3·5 cm.

Posture and intrapleural pressures. Variations of intrapleural pressure with posture were studied in both rabbit and cat under light and deep ether anæsthesia. The *rabbit*, with a more fragile bony thorax, and a thinner, and much less muscular, diaphragm, responded with a much greater variation than did the cat. Average readings are shown in Table I.

TABLE I. Posture and intrapleural pressures, inspiratory : expiratory. Average pressures in cm. water

Animal	State	Horizontal (dorsal)	Vertical (head up)	Inverted (head down)	Number of animals included in average
Rabbit	Light ether	−0·5 to −3·5	−2·5 to −4·5	+0·5 to −3·0	8
	Respiratory arrest by ether	−0·75	−2·5	+0·75	7
Cat	Light ether, chloralose	−1·0 to −2·0	−1·5 to −2·5	0 to −1·5	4
	Respiratory arrest by ether	−1·0	−2·0	−0·5	4

With the head held up the expiratory negative pressure is increased, probably by traction on the diaphragm as the abdominal viscera tend to fall away from it. Inspiratory pressures alter in the same direction. When inverted, the rabbit is unable to maintain a negative pressure in expiration. Respiration becomes embarrassed and rapid, inspiration is effected with effort, so that inspiratory pressures are almost as great as usual. They may even be greater; in one instance an excursion of from −1·5 to −3·5 cm. in the horizontal position became one of 0 to −6 cm. when the head was lowered.

When respiration was arrested by deep ether anæsthesia, a negative pressure resulted in the dorsal and head-up postures; but a positive pressure (0·25–1·5 cm.) was obtained when the animal was inverted.

The *cat* shows similar but smaller variations of pressure with varying body posture. A negative pressure is usually maintained on arrest of respiration by ether anæsthesia, even in the inverted position.

Curarine and intrapleural pressures. In uncomplicated curarization, respiration is arrested as a result of paralysis of the respiratory muscles. X-ray observations showed that in both rabbits and cats respiration is largely longitudinal (i.e. diaphragmatic) in type, and also showed paralysis

of the diaphragm as the chief factor in respiratory failure from curarization. Records of intrapleural pressures show that, with this paralysis of the diaphragm, the curarized *rabbit* may fail to maintain a negative pressure, even in the horizontal position. In some cases the diaphragm loses its "tone" to such a degree that positive pressures as high as 2 cm. water are recorded. Otherwise, and throughout in the *cat*, the intrapleural pressures in curarization resembled those of fatal anæsthesia (Table II).

TABLE II. Curarine and intrapleural pressures. Average figures

Animal	Horizontal	Head up	Head down	Number of animals included in average
Rabbit	0	−1·25	+1·0	4
Cat	−1·5	−2·0	−0·75	3

Retraction of the exposed lung: bronchial spasm

Failure to maintain intrapleural "negative" pressure might be solely responsible for the collapse of the lung found at post-mortem in curarine poisoning. Reduction of intrathoracic volume would produce a relative broncho-constriction, and this might perhaps be alleviated by adrenalin in its broncho-dilator function. The direct bronchial action of curarine was therefore studied upon the exposed lung, and for this purpose guinea-pigs were chosen. They suffer from curarine respiratory "spasm", and in addition are notoriously sensitive to the true bronchial spasm produced by histamine.

Exposed lung in pithed guinea-pig. The lungs of pithed guinea-pigs were exposed from below by opening the abdomen widely and incising the diaphragm. The lungs were ventilated by a pump, under a measured air-pressure, and injections made direct into the right ventricle of the exposed heart. Curarine in doses which varied from 0·1 mg. upward, caused the lungs to retract, decrease the amplitude of their movement, and blanch. The lungs appear to lose their elasticity, and the minimum pressure required for distension rises. The action resembles and yet differs from that of histamine. Whereas 0·1 mg. of histamine completely arrests lung movement by its action on the bronchial muscles, and smaller doses cause a temporary decrease in lung movement, large doses of curarine were never found to abolish expansion of the lung completely, at any normal air pressure. Again, while small doses of histamine (0·02 mg.) cause a slight retraction and a diminution of lung movement resembling that caused by curarine, with the latter the lung surface

becomes a greyish white colour which coincides in onset with a severe general cyanosis absent in the comparable histamine experiments. The appearance is as if effective oxygenation ceased at a lesser reduction of lung movement with curarine than with histamine (Table III).

TABLE III. Minimal lung distension pressures

Initial pressure cm. H_2O	Drug	Resultant pressure cm. H_2O	Condition of lungs	Effect of adrenaline
1–2	Histamine 0·02–0·1 mg.	Over 10 (usually very high)	Respiration arrested	Re-expansion with large doses
1–2	Curarine 0·1–0·5 mg.	7–10 (never very high)	Lung retracted. Blanching of surface. Expansion reduced	Retraction removed. Blanching removed. Re-expansion with large doses

These appearances were taken to indicate that curarine causes a bronchial spasm. The spasm resembles that caused by histamine. But it was difficult to attribute the blanching of the lung, and the high degree of cyanosis which occurs (when the lung is retracted only some 10 or 20 p.c. and still has considerable movement) merely to the weaker action of curarine. It is possible that curarine has a greater action, proportionately, on the minute terminal bronchioles than has histamine.

The bronchial spasm caused by curarine has a response to broncho-dilator drugs similar to that of histamine. Intracardiac adrenaline (0·1 c.c. of 1 : 1000) causes the lungs to re-expand instantaneously, and to become pink of hue. The effect is transitory, lasting 1 or 2 min. only. Atropine must be given in large doses to have any dilator effect: its action is then prolonged. Large preliminary doses of atropine protect against the spasm.

Retraction of the lung in situ. Guinea-pigs were used as in the above experiments. But instead of the lungs being exposed, they were viewed through the transparent diaphragm, after opening the abdominal cavity. At the onset of the curarine spasm the bases of the lungs withdraw slightly, diminishing the area of their contact with the diaphragm. As the diaphragm itself rises into the chest cavity as a result of its loss of "tone", this further shrinking of the lungs must represent a very considerable decrease in lung volume. Further, it appears that, at least in these animals, loss of the suction normally applied to the lungs by the "negative" intrathoracic pressure, combined with the broncho-constrictor action of curarine, and whatever natural elastic "recoil" the lungs themselves may possess, causes the separation of the lungs from the chest walls, and a corresponding increase in the size of the pleural cavity.

Discussion

The collapse of the lungs which is characteristic of curarine poisoning is brought about by a combination of the factors considered below. (1) *Curarization of the muscles of respiration*, and particularly of the diaphragm, leads to a reduction of the normal "negative" pressure in the pleural cavity, and a consequent reduction of the volume of the thoracic cavity (Figs. 1 and 2). The whole lung is reduced in size, and, in as far as the "negative" intrapleural pressure can be regarded as broncho-dilator in effect, broncho-constriction may be regarded as an element of respiratory curarization proper. In the rabbit, which lacks a rigid chest wall and a powerful diaphragm, the effect of curarine is to abolish the "negative" intrapleural pressure, unless the animal is suspended vertically from the head. But in the more strongly built cat, the reduction of negative intrapleural pressure produced by curarine does not exceed that produced by arrest of respiration by ether anæsthesia (Tables I and II). Thus a fall of intrapleural negative pressure, produced by curarization of the respiratory muscles, does not alone explain the difficulty of artificial respiration by external compression in certain postures. For these changes of intrapleural pressure occur with ether arrest of respiration, and the difficulty of artificial respiration does not. (2) *Posture* measurably affects intrapleural pressures in normal cats, and greatly alters them in rabbits. It is probably this effect of posture which renders artificial respiration by compression possible in curarized animals held vertically with the head up, when it has become ineffective in the horizontal position (Table II). Curarine further causes (3) *active retraction of the lung*, probably as a result of a constricting action on the smaller bronchi, as is evidenced by the observations on exposed lungs in guinea-pigs. This effect is antagonized by adrenaline and by atropine in large doses.

The broncho-constrictor action of curarine resembles, but is not identical with, the action of histamine on the lung (Table III). Large doses of curarine never produce the intense histamine bronchial spasm, while small doses of histamine seem to produce less blanching of the lung surface, and less general cyanosis, compared with the reduction of lung movement than does curarine. The general pharmacological action of curarine does not show resemblances to that of histamine, for instance upon blood pressure or intestinal movements [West, 1937]. Curarine bronchial spasm has been recorded in man [West, 1936]. It was not accompanied by flushing of the skin, nor did the blood pressure fall.

The fact that the broncho-constrictor action of curarine is associated

with inspiratory difficulty and pulmonary collapse, instead of with the expiratory difficulty and pulmonary distension which occurs with broncho-constriction from other causes (e.g. histamine, asthma), may be attributable to two causes: (1) the weakness of power of contraction, and of maintenance of contraction, of the respiratory muscles produced by curarine; (2) the factors, tending towards pulmonary collapse enumerated above, and which result from a loss of "tone" in the muscles of respiration and particularly in the diaphragm. Both causes are properties of normal curarization as it affects these particular muscles.

Curarine has come to be regarded as the characteristic active principle of the "calabash" Macusi curare of British Guiana. It has been prepared both from this "calabash" curare and direct from its plant ingredient, *Strychnos toxifera* [Boehm, 1895; King, 1935]. Boehm attributed to it the formula $C_{19}H_{26}ON_2$. Both Boehm and King noted that this alkaloid was amorphous; it could never be obtained in crystalline form. Recently, Wieland *et al.* [1937] have published an investigation of a "calabash" curare from which they have isolated an alkaloid "Toxiferin", of a curariform potency many times that of curarine, as judged by its minimal lethal dose in the frog. Further reports of the pharmacological properties of this new alkaloid have not yet been communicated, but its potency renders it a more acceptable essential alkaloid of Guianese curare than curarine has proved to be. It will be of interest if this new alkaloid proves to be obtainable direct from *S. toxifera*. It is possible that it may prove not only more potent in its curariform action, but purer, and in particular, free of the broncho-constrictor action of curarine.

Summary

An enquiry into the actions of curarine upon the respiratory mechanism was undertaken, because it causes a sudden fatal respiratory embarrassment, which can be observed in the absence of full general curarization, and which is characterized by (i) an apparent bronchial spasm, (ii) the failure of artificial respiration by chest compression, and (iii) a tendency to collapse of the lung as a post-mortem finding.

The action of curarine on the respiratory mechanism may be summarized as follows:

There is a direct constrictor action upon the bronchi and bronchioles. Normally this would probably lead to expiratory dyspnœa, but this effect of broncho-constriction is prevented by the other action of curarine, which is to curarize the muscles of respiration. Partial curarization produces two effects upon muscle: there is (*a*) a reduction

in the power of muscular contraction, particularly in the power of maintained (i.e. tetanus) contraction, and (*b*) a reduction in the normal tone of the muscle. When these two actions of curarine affect the respiratory muscles, and, in particular the diaphragm, they cause respectively, (*a*) a weakened and poorly maintained inspiratory effort, (*b*) a reduction of the thoracic volume (Figs. 1 and 2) and a fall in the normal "negative" intrapleural pressure (Table II). The result is a partial collapse of the lung. It is even possible that the reduced intrapleural pressure resulting from loss of muscular tone in the diaphragm, assisted by the normal elastic recoil of the lungs, may convert the "potential" pleural cavity, which probably normally contains a little air, into an "actual" cavity, the lungs shrinking away from the chest-wall. Finally reduction of negative intrapleural pressure causes a *passive* broncho-constriction.

The cause of the sudden failure of respiration in curarization with curarine is thought to be acute pulmonary collapse. The sequence of events is: (1) loss of tone in the respiratory muscles, particularly in the diaphragm; (2) consequent reduction in chest volume, and fall in intrapleural negative pressure. The resulting retraction of the lung is assisted by (3) an active broncho-constriction produced by curarine, (4) the passive broncho-constriction produced by a rise in intrathoracic pressure, and (5) a loss of power of contraction and a failure of maintenance of contraction in the inspiratory muscles.

Of these factors the active broncho-constriction (3) may be due to the presence of an impurity. The recent report of the isolation for the first time of a crystalline alkaloid ("Toxiferin") from calabash curare [Wieland *et al.* 1937] suggests that the amorphous curarine hitherto isolated may be impure. "Toxiferin" is reported to have a curarizing power many times greater than that of curarine.

I am indebted to Dr J. W. Thornton for his collaboration in the lung-perfusion experiments mentioned in the introduction to this Paper; to the Director and Assistant Directors of the Nuffield Institute of Medical Research, Oxford, for the X-ray facilities referred to; to Dr K. J. Franklin for the loan of apparatus and for suggestions, and to Sir Henry Dale for advice throughout the work, which was done while in receipt of an Expenses Grant from the Medical Research Council.

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THE RELATION OF THE HYPOPHYSIS TO CHANGES IN SUGAR TOLERANCE AND INSULIN SENSITIVITY INDUCED BY CHANGES OF DIET

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In a series of investigations concerning the effect of diet on carbohydrate metabolism it was found that a definite correlation existed between the sugar tolerance and the sensitivity of the animal to insulin. It was demonstrated, both in man and in rabbits, that diets which caused an improvement in sugar tolerance also permitted a more rapid depression of the blood sugar by insulin, whilst diets which produced a diminished sugar tolerance always retarded the rate at which the blood sugar fell after insulin [Himsworth, 1933, 1934*a*, *b*]. Subsequently it was found that, in healthy man, the only dietetic factor influencing sugar tolerance and insulin sensitivity was the absolute amount of carbohydrate in the preceding diet; low carbohydrate diets decreasing, and high carbohydrate diets increasing, both sugar tolerance and insulin sensitivity. In the same paper [Himsworth, 1935–6*a*] it was also shown that the changes in the blood sugar tolerance curve, in response to change of diet, could be completely accounted for by coincident changes in sensitivity to the endogenous insulin secreted in response to the rise of blood sugar induced by the test dose of glucose. It seemed to us that an explanation of these results might be found through the work of Cope & Marks [1934–5] and Young [1936] which showed that an extract of the anterior lobe of the hypophysis retarded, in healthy animals, the rate and the degree of depression of the blood sugar after injection of insulin. The resemblance between their insulin depression curves on animals treated with this extract and the insulin depression curves found after administration of a low carbohydrate diet was sufficiently marked to suggest

that the hypophysis might play a part in the variations in insulin sensitivity and glucose tolerance induced by dietary changes. The experiments reported in this paper were undertaken to test this hypothesis.

Methods

Hypophysectomy. Male rabbits 2 kg. in weight were used. The hypophysis was removed through the buccal route by the method originally described by White [1933]. In respect of these operations we were greatly helped by Dr Oliver Cope of Boston, U.S.A. and Dr W. H. Newton of University College, London, who communicated to us various technical modifications which greatly facilitated the operative technique. Pituitary gland tissue was identified histologically in each case in the debris removed at operation.

Diet. The low carbohydrate diet consisted of 50 g. of fresh cabbage and 120 g. of extracted soya bean meal. The high carbohydrate diet was composed of approximately 250 g. of cabbage, 150 g. of oats and bran mixed in equal proportion and 20 g. of sucrose mixed with the oats and bran. The approximate daily composition of the low carbohydrate diet was soluble carbohydrate 12 g., protein 50 g., fat a trace, cellulose 36 g.; of the high carbohydrate diet soluble carbohydrate 90 g., protein 18 g., fat 7 g., cellulose 50 g.

A word of explanation is necessary with regard to the use of extracted soya bean meal. It arose from an accident. When the original work on the correlation of sugar tolerance and insulin sensitivity in rabbits was undertaken by one of us [Himsworth, 1934*a*] he was under the impression that the controlling dietary factor was the amount of fat in the diet. Soya bean meal was thought to be a useful basis for such a diet. The first samples supplied contained 17 p.c. of fat. At some time early in the course of the investigations, an extracted soya bean meal, which contains only a trace of fat, chanced to be substituted and this substitution was not discovered until after the paper [Himsworth, 1934*a*] recording the results had been published. It was ultimately proved that neither the amount of fat, nor the amount of protein, nor the calorie value of the diet, but only the absolute amount of dietary carbohydrate influences the sugar tolerance and the insulin sensitivity tests [Himsworth, 1935–6*a*], and the explanation as to why these tests were unaffected by the substitution of extracted soya bean meal was apparent. When the present investigations were undertaken it was decided to use the same diet which previously had provoked such marked decreases in sugar tolerance and insulin sensitivity.

Anterior pituitary extract (*glycotropic factor*). This extract was generously given to us by Dr F. G. Young of the National Institute for Medical Research, Hampstead. Fractionation studies by Young [1937*a*] have shown that the active substance in this extract is distinct from the gonadotropic, thyrotropic and lactogenic principles. The details of this work will be published shortly by Dr Young.

Sugar tolerance. It was shown in a previous paper [Himsworth, 1934*a*] that the sugar tolerance of a rabbit was more clearly revealed by the effect on the blood sugar of four consecutive intravenous injections of 1 g. of glucose at intervals of half an hour, than by following the effect of one intravenous dose of glucose for a period of one or more hours. 5 c.c. of a 20 p.c. solution of glucose in normal saline were injected in the course of 1 min. at intervals of 30 min. Samples of blood for sugar estimation were taken from the ear vein at 6 and 28 min. after each injection.

Insulin sensitivity. This was tested by finding the effect on the blood sugar of injecting intravenously ½ unit of crystalline insulin. Further details of the technique employed in this and the sugar tolerance tests have been given elsewhere [Himsworth, 1934*a*].

Blood sugar. This was estimated in 0·1 c.c. of whole blood by the Hagedorn-Jensen method.

Preparation of animals. Recently hypophysectomized animals develop fatal hypoglycæmia if starved or deprived of carbohydrate, but after several months they become less sensitive to such deprivation.

In this investigation twenty-four rabbits survived operation; six died within 10 days of operation from hypoglycæmia referable to refusal of food, and a further ten died, presumably from the same cause, during the attempt to habituate them to a low carbohydrate diet. All these animals showed no macroscopic remains of pituitary tissue at post-mortem. No success was achieved in keeping the animals alive on a low carbohydrate diet until 6 months had elapsed after hypophysectomy. Up to this time the animals were given the high carbohydrate diet, and it was then found possible, over the course of 3 or 4 weeks gradually to withdraw carbohydrate and substitute the foodstuffs of the low carbohydrate diet. The sugar tolerance and insulin sensitivity of each animal was tested first whilst taking the high carbohydrate diet, then on the low carbohydrate diet, and finally again on the high carbohydrate diet. The animals were then killed and serial sections of the base of the brain and sella turcica made. In every one of the eight animals that survived the dietary change remnants of pituitary tissue were found in the region of the stalk.

The presence of these hypophyseal remnants would ordinarily be an indication for discarding the results, but the striking differences in the reaction to diet between these animals and the unoperated control rabbits justifies us in our opinion in not taking this course. Further, we think it probable that this series of experiments could not be performed on completely hypophysectomized animals as these would almost certainly die when given a low carbohydrate diet.

The pituitary remnants varied in size. In three animals at post-mortem none were visible, but histological examination revealed islets of half a dozen or so cells surrounded by fibrous tissue in the region of the pituitary stalk. Despite this, these animals showed marked atrophy of the testes and suprarenals and two had the large deposits of intra-abdominal fat noted by Cope & Marks [1934–5] in their hypophysectomized rabbits. In four other animals pieces of pituitary-like tissue about the size of a pin's head were seen at post-mortem and their nature confirmed histologically. These animals showed no atrophy of other glands. It is worthy of note that in these seven animals the pituitary tissue remaining showed histologically small shrunken cells which stained poorly. The eighth rabbit was found to have had only a small portion of its pituitary removed and the major portion of the gland was found to have been displaced into the cranial cavity. This animal provided an excellent control for it reacted to dietary changes in exactly the same way as did the normal rabbits used as controls.

Evidence that after destruction of the pituitary gland new glandular tissue may arise from cell rests in the base of the brain has been brought forward by Cameron [1924]. In view of the fact that our animals were allowed to survive up to a year after operation, the question arises as to whether the portions of pituitary tissue found at post-mortem were pieces of the gland which had escaped removal at operation or were regenerating glandular tissue. This question unfortunately cannot be decided now. It is perhaps relevant that our animals showed grossly impaired sugar tolerance at the end of 2 weeks after operation, and this, in view of the subsequent spontaneous improvement to normal tolerance during the next month, might indicate that remnants of tissue had been left behind and that these were stimulated into over-activity by the inflammation resulting from the operative procedures.

The question will naturally be asked whether the phenomena observed 6 months to 1 year after operation can be attributed to removal of the hypophysis or to derangements in function of other endocrine glands as a result of changes occurring in them secondarily to hypophysectomy. This point cannot be answered definitely, but our results concerning sugar tolerance and insulin sensitivity were the same whether secondary changes such as degeneration of the adrenals or testes were marked or not, and further, where our investigations repeat the experiments of other workers on hypophysectomized animals, the results are in accord with the majority of reports.

All the experiments recorded in this paper were performed at 6 months

to 1 year after hypophysectomy. The animals were fed on the day prior to the test and food was allowed to stay in the cage. By the late evening this had always been consumed, and no further food was given until after the test.

On the day of the test the rabbits were brought down to the laboratory, warmed, and 2 hr. later placed in a warm box. They remained in the box until the experiment was ended.

In the experiments in which Young's glycotropic factor was used this was given in two doses, each corresponding to 2 g. of fresh anterior pituitary gland. One dose was given at 6 p.m. the previous evening and the second at 9 a.m. on the day of the experiment.

Tests were started at 11 a.m. save in the case of experiments with anterior pituitary extract when the test commenced at 12 noon.

Results

Effects of change of diet

In the normal animal the change from a high to a low carbohydrate diet results in a decrease of sugar tolerance (Fig. 1) and a decrease in sensitivity to insulin (Fig. 2). In the hypophysectomized animal the same change in composition of the diet produces little effect upon either the sugar tolerance (Fig. 3) or the sensitivity to insulin (Fig. 4). This abolition of the response to diet was found in each of the seven rabbits in which the hypophysis had been almost completely removed. The eighth—that in which a practically intact hypophysis had been displaced into the cranial cavity—reacted to the change in diet as did the normal control animals.

On closer inspection of the two figures showing the results on the hypophysectomized animals, it will be seen that on the low carbohydrate diet the successive blood sugar curves rose higher than when the high carbohydrate diet was being taken. Similarly on the former diet the curve of depression of the blood sugar after insulin fell in the early part of its course slightly more slowly than did the curve during the high carbohydrate regime. In view of the fact that some small remnants of hypophysis were found at the post-mortem examination of these animals, we do not feel justified in attaching importance to these findings. One point, however, is worthy of note. When the hypophysectomized animals were taking the high carbohydrate diet they showed the Staub-Traugott effect; that is, each consecutive injection of glucose resulted in a smaller rise of blood sugar than the previous one.

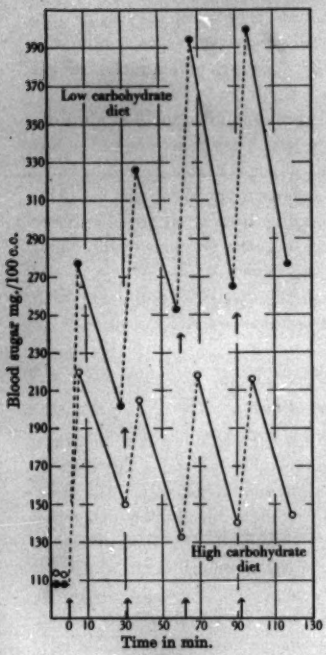

Fig. 1.

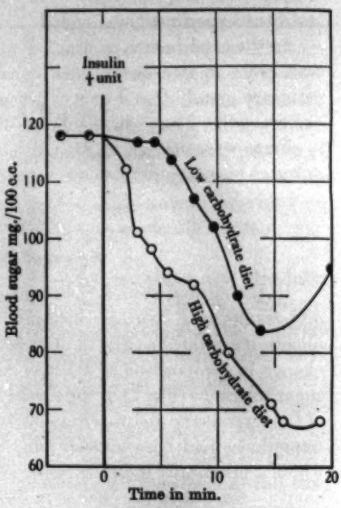

Fig. 2.

Fig. 1. Two series of consecutive glucose tolerance curves from the same *normal* animal after intravenous injection of 1 g. of glucose every 30 min., the one (disks) obtained on the low carbohydrate diet, the other (circles) obtained on the high carbohydrate diet. The times of glucose injection are indicated by arrows.

Fig. 2. Two insulin depression curves from the same *normal* animal, one (disks) obtained on the low carbohydrate diet, the other (circles) obtained on the high carbohydrate diet. Resting blood sugar level before experiment whilst on low carbohydrate diet 118 mg./100 c.c.; whilst on high carbohydrate diet 118 mg./100 c.c.

Effect of anterior pituitary extract (glycotropic factor)

As removal of the pituitary gland abolishes the impairment of sugar tolerance and of insulin sensitivity which results from a low carbohydrate diet, the next step in the investigation was to discover if the administration of an extract of the anterior lobe of the pituitary to hypophysectomized rabbits taking a *high* carbohydrate diet would result in an

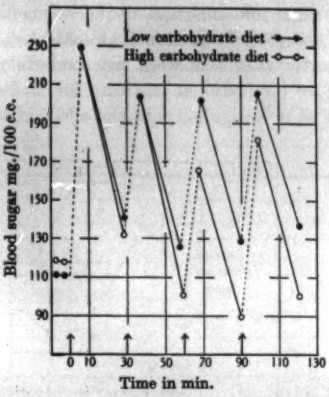

Fig 3. Two series of consecutive glucose tolerance curves, after intravenous injection of 1 g. of glucose every 30 min., from the same *hypophysectomized* animal, one (disks) obtained whilst the animal was receiving a low carbohydrate diet, the other (circles) whilst receiving a high carbohydrate diet. The times of glucose injection are indicated by arrows.

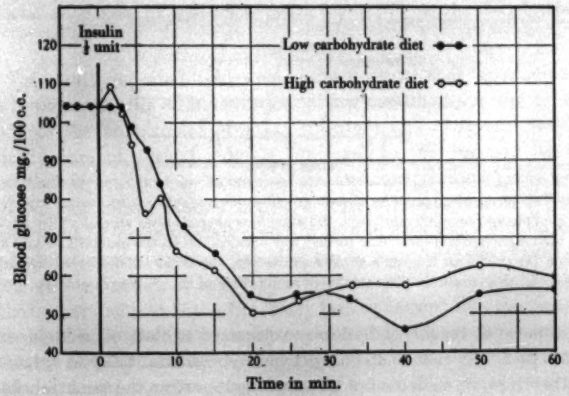

Fig. 4. Two insulin depression curves, after intravenous injection of ½ unit of insulin, obtained from the same *hypophysectomized* animal, one (disks) whilst on a low carbohydrate diet, the other (circles) whilst on a high carbohydrate diet. Resting blood sugar level on low carbohydrate diet 114 mg./100 c.c.; on high carbohydrate diet 116 mg./100 c.c.

impairment of its sugar tolerance and also of its insulin sensitivity comparable with that observed in the normal animal when given a *low* carbohydrate diet. Shortly after this work was undertaken we learnt that Dr F. G. Young, of the National Institute for Medical Research, had almost completed an investigation into the effect of such an extract on

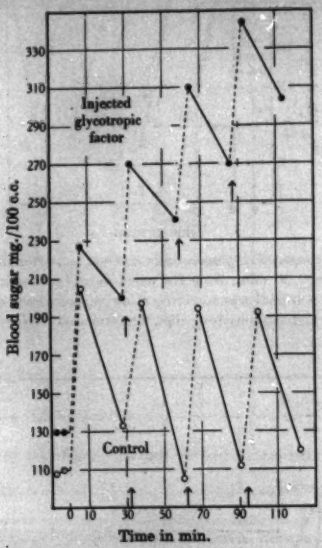

Fig. 5. Two series of consecutive glucose tolerance curves, after intravenous injection of 1 g. of glucose every 30 min., from the same *hypophysectomized* animal whilst receiving a high carbohydrate diet. One (disks) was obtained when the animal had previously been injected with Young's glycotropic factor, the other (circles) when it had not received this substance. The arrows show the time of the glucose injections.

the response to insulin of hypophysectomized rabbits on a high carbohydrate diet. Our results and his proved to be substantially in agreement and, therefore, we shall confine ourselves to reporting the essential change observed by us, whilst the detailed experiments will be published by Dr Young.

After administration of the extract of the anterior lobe of the pituitary to the hypophysectomized rabbit the sugar tolerance was greatly impaired

(Fig. 5). It was also proved, in agreement with Dr Young, that the sensitivity to insulin markedly decreased (Fig. 6).

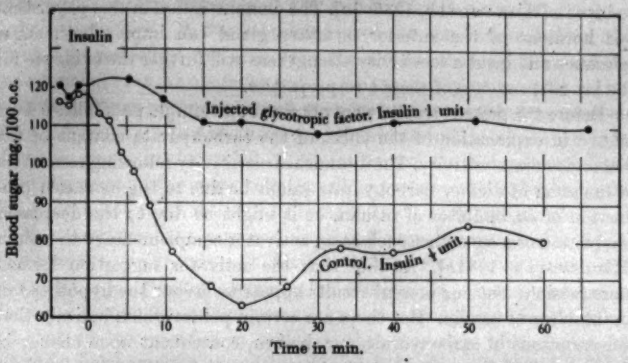

Fig. 6. Two insulin depression curves, after intravenous injection of insulin, on the same *hypophysectomized* animal receiving a high carbohydrate diet. One (disks) was obtained when the animal had previously been injected with Young's glycotropic factor and in this case 1 unit of insulin was injected; the other (circles) when it had not received this substance and in this case ½ unit of insulin was injected. Resting blood sugar level after Young's glycotropic factor 119 mg./100 c.c.; in control experiment 116 mg./100 c.c.

Discussion

The results reported in this paper demonstrate that, after removal of the hypophysis, the administration of low carbohydrate diet no longer produces the impairment of sugar tolerance and of insulin sensitivity which occurs in normal animals subjected to this regime. Also they show that injection of an extract of the anterior pituitary gland into the hypophysectomized rabbits taking a high carbohydrate diet produces impairment of sugar tolerance and of insulin sensitivity similar to that produced in normal animals by a diet low in carbohydrates. These results prove that a functional activity of the hypophysis is controlled by the carbohydrate content of the diet. They further suggest that this functional activity is such that the hypophysis reacts to limitation in the amount of available carbohydrate by increasing its secretion of a substance, similar to or identical with the glycotropic factor of the anterior pituitary, and thus causes impairment of sugar tolerance and decrease of sensitivity to insulin.

In a previous series of publications the correlation between sugar tolerance and insulin sensitivity has been established [Himsworth, 1934*a*, *b*], and it has been shown that the changes in sugar tolerance

consequent upon variation in the amount of dietary carbohydrate can be completely accounted for by changes in the sensitivity of the organism to insulin [Himsworth, 1935–6*a*]. The demonstration in this paper that one hormone of the anterior pituitary gland can impair both sugar tolerance and insulin sensitivity strengthens still further the evidence for the interdependence of these two properties.

Before the present results were obtained two simple possibilities were offered in explanation of the effect of the carbohydrate content of the diet on insulin sensitivity. The diminished sensitivity following restriction or omission of dietary carbohydrate might be due to the increased production of an inhibitor of insulin, or it might be due to the decreased production of a substance such as an activator complementary to insulin [Himsworth, 1934*a*]. At that time the activator suggestion seemed more feasible, but our present results appear to favour the hypothesis of an inhibitor of insulin. But there are certain points which indicate that the variations in carbohydrate metabolism, consequent upon change in the amount of dietary carbohydrate, may be the result of mechanisms considerably more complex than a simple change in the rate of output of an inhibitor of insulin.

It has been pointed out by one of us [Himsworth, 1934*a*] that the specific dynamic action of glucose in an animal varied in parallel with the sugar tolerance and sensitivity to insulin. It is diminished by restriction of and increased by liberal administration of carbohydrate. In 1930 Dann & Chambers showed on dogs that after 3 weeks' starvation, the rise of R.Q. which normally follows the administration of glucose could no longer be obtained, but that the giving of glucose to the starved animals on 5 successive days restored the rise of R.Q. to its normal magnitude. Chambers *et al.* [1935] have now shown that in hypophysectomized dogs glucose caused the usual rise of R.Q., and that after starvation these dogs show the same diminished rise of R.Q. following glucose administration as do starved normal dogs. These observations that in hypophysectomized animals restriction of carbohydrate still abolishes the specific dynamic action of glucose whilst it no longer causes impairment of insulin sensitivity and glucose tolerance suggest that some other mechanism, as well as the one now demonstrated, is involved in the reaction of the animal to carbohydrate deprivation.

There is a further piece of evidence in support of this view. In 1919 Hamman & Hirschman showed that if the same dose of glucose were given at intervals of 90 min. the blood sugar tolerance curve improved with each successive dose. This phenomenon is now usually referred to as

the Staub-Traugott effect. It has been shown [Himsworth, 1933, 1934*a*] that after several doses of glucose an improvement in sensitivity to insulin also occurs, but only if the animal tested is receiving a high carbohydrate diet. It was suggested that the mechanism behind the Staub-Traugott effect was essentially the same as that which caused the improvement in sugar tolerance and insulin sensitivity when a high carbohydrate diet was given. It should, therefore, be impossible to obtain this effect in hypophysectomized animals. Reference to Fig. 5 will show that, although change in the carbohydrate content of the diet produces little or no alteration in the sugar tolerance of hypophysectomized rabbits, the Staub-Traugott effect can still be demonstrated on these animals.

Most workers have reported improved sugar tolerance after hypophysectomy. Sakamoto & Saito [1932]; Fujimoto [1932] and Corkhill *et al.* [1933–4], working on rabbits, support this statement. From our results we are not prepared to offer an opinion on this point. All that we feel justified in saying is that the sugar tolerance of our rabbits, after operation, was as good as that of normal rabbits receiving a high carbohydrate diet.

The results which we have presented in this paper suggest an interesting possibility with regard to the causation of human diabetes mellitus. Young [1937*b*] has recently published some remarkable experiments showing that after injection into normal dogs of an extract of the anterior pituitary gland a state of permanent diabetes can be produced. It is noteworthy that the disease thus produced resembles human diabetes mellitus more closely than does the diabetes of depancreatized dogs. Our results indicate that a low carbohydrate diet stimulates the pituitary gland to increased activity. There is some evidence that the diets of diabetic patients before the onset of the disease are relatively low in carbohydrate [Himsworth & Marshall, 1935–6]; and there is a great deal of evidence suggesting that the incidence of diabetes mellitus is high in countries and under circumstances in which *low* carbohydrate diets are eaten, whilst the incidence is low where and when *high* carbohydrate diets prevail. In explanation of these findings it has previously been suggested that, whilst heredity was the factor predisposing to the disease, the habitual ingestion of a low carbohydrate diet was the factor which precipitated diabetes mellitus [Himsworth, 1935–6*b*]. If the taking of a low carbohydrate diet could be shown to stimulate the pituitary gland to over-secretion, not only of the glycotropic factor, but of the closely associated substance which produces diabetes mellitus in dogs, then the suggestion made on clinical grounds would be justified.

SUMMARY

1. Normal rabbits receiving a low carbohydrate diet show impaired sugar tolerance and diminished sensitivity to insulin. On giving a high carbohydrate diet the sugar tolerance increases and the sensitivity to insulin increases.

2. In hypophysectomized rabbits this reaction to the change of diet is abolished. The sugar tolerance and insulin sensitivity remain high whether a low or a high carbohydrate diet is given.

3. In hypophysectomized rabbits taking a high carbohydrate diet, injection of Young's glycotropic factor of the anterior lobe of the pituitary gland results in impairment of sugar tolerance and of sensitivity to insulin similar to that observed when normal animals are given a low carbohydrate diet.

4. These results show that a functional activity of the hypophysis is influenced by the composition of the diet. The impairment of sugar tolerance and of insulin sensitivity resulting from administration of a low carbohydrate diet may well be explained by such a diet stimulating the hypophysis to secrete in increased amounts a substance identical with or similar to Young's glycotropic factor. It is indicated, however, that other factors may also be concerned in the effect produced by low carbohydrate diets.

5. The bearing of the results on certain aspects of physiological and pathological carbohydrate metabolism is discussed.

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THE ACTION OF TYRAMINE AND ADRENALINE ON THE DENERVATED NICTITATING MEMBRANE

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In the course of examining the action of cocaine on physiological responses to adrenaline and tyramine, Burn & Tainter [1931] made the observation that tyramine has no dilator action on the denervated pupil of the cat's eye. Their experiments were performed 9 and 16 days after removal of one superior cervical ganglion, using urethane as an anæsthetic; doses of 0·5 mg. tyramine, injected intravenously, caused the normal pupil to dilate, while doses as large as 8·0 mg. had no action on the denervated pupil. Later Burn [1932] showed that this was also true of the iris when isolated in a bath of Ringer, though the isolated iris is a relatively insensitive organ; he further investigated the action of tyramine on the blood vessels of the cat's foreleg and found that after removal of the stellate ganglion and subsequent degeneration of the sympathetic fibres, tyramine failed to cause constriction of the foreleg, although constriction occurred in the opposite normal foreleg.

Similar differences between the action on the denervated and on the innervated structures were found for ephedrine, though large doses of ephedrine (10 mg.) had a slight dilator action on the denervated pupil.

Since the effect of adrenaline on the pupil is increased by denervation and that on the blood vessels is at least not appreciably diminished, Burn argued that a qualitative difference must exist between the point of action of tyramine and that of adrenaline; he drew the conclusion that tyramine in general must act on the sympathetic nerve ending itself, so that after denervation there was no longer a site of action for it. Recently, however, Bacq [1936] has described the results of examining tyramine and other substances on the denervated nictitating membrane; he found that, as with adrenaline, the effect of tyramine was greater on the

denervated than on the normal membrane. So far as can be judged, the number of observations made with tyramine was not great, since Bacq tested very many compounds; his experiments were performed with dial as anæsthetic. Because his result was so completely at variance with those on the pupil and the vessels of the forelimb, we have examined the action of tyramine ourselves, and also that of dioxyphenylethylamine, sympatol and adrenaline. Sympatol has the same structure as adrenaline except that there is only one —OH group in the ring; dioxyphenylethylamine has the same structure as tyramine except that there are two —OH groups in the ring.

Experimental methods

The removal of the superior cervical ganglion of one side was performed at different periods varying from 1 to 75 days before the final experiment. In the final experiment the cat was anæsthetized with ether, the abdomen was opened and the suprarenal glands were excluded from the circulation by ligatures; the abdomen was closed. Artery clips were then placed temporarily on both carotid arteries, the spinal cord was divided at the second cervical vertebra and the brain was destroyed. The foramen magnum was plugged with plasticine and a cork. Ether was then discontinued, and the cat artificially respired. The clips on the carotid arteries were removed.

To record the contractions of the nictitating membranes the head of the cat was fixed rigidly in a clamp, and the outer corners of the lids were incised. A silk thread through the middle of each membrane passed by way of a pulley wheel to an isotonic lever with a frontal writing point. The levers were of equal length and weight, and the threads were attached at the same distance from the fulcrum. The blood pressure was recorded from the femoral artery of one side, and a cannula for injection was inserted into the femoral vein of the other. The cervical sympathetic chain of the unoperated side was divided to exclude impulses reaching the ganglion from the spinal cord.

I. *Results with tyramine*

The characteristic response. At the outset of the experiments it did not seem likely that the results could be other than simple; to our surprise, however, this was not so; they varied from one experiment to another, and it is not easy to select one as typical of all the rest. We begin, however, by describing the experiment shown in Fig. 1 which is characteristic of tyramine in that similar records have never been

obtained with adrenaline, dihydroxyphenylethylamine or sympatol. The experiment was carried out 24 days after extirpation of the right superior cervical ganglion. When 0·4 mg. tyramine (i.e. 0·66 mg. tyramine acid phosphate) was injected intravenously, there was no effect on the normally innervated membrane, but a small contraction of the denervated membrane took place which was delayed in onset and prolonged. This is seen in Fig. 1 (*a*). At this point the denervated membrane was more sensitive than the normal. In Fig. 1 (*b*) is shown the action of 0·8 mg. tyramine. A contraction occurred in the denervated membrane, slightly earlier than a contraction of the same height, though of shorter duration in the normal membrane. In Fig. 1 (*c*) and (*d*) are shown the

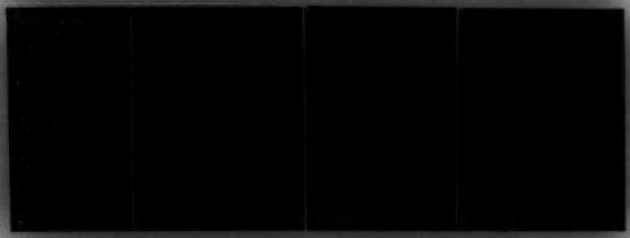


Fig. 1. Contractions of nictitating membranes of spinal cat. The superior cervical ganglion was excised on the right side 24 days previously. In (*a*) a dose of 0·4 mg. tyramine causes contraction of the denervated membrane, but not of the normal. In (*c*) and (*d*) doses of 1·6 and 4·0 mg. tyramine cause a much greater contraction of the normal membrane than of the denervated.

effects of higher doses of tyramine, namely 1·6 and 4·0 mg.; these effects are strikingly different, for in each the contraction of the normal membrane is greater than that of the denervated membrane. The contractions of the denervated membrane in response to the different doses are all very much alike, showing little increase in size in proportion to the dose; they are, moreover, prolonged contractions. The contractions of the normal membrane increase rapidly in size according to the dose, and they are contractions of short duration.

Variations according to the interval after ganglionectomy. In one experiment only did tyramine fail to stimulate the denervated membrane in any dose, and in all others the lower doses made the denervated membrane contract without causing a contraction of the normal membrane; this sensitization of the denervated membrane is illustrated in Fig. 2, which in (*a*) shows the response in a cat 2 days after ganglionectomy,

and in (*b*) shows it in a cat 75 days after ganglionectomy. These results resemble the observation of Bacq already mentioned.

Fig. 2. Upper record, denervated nictitating membrane; lower record, normal membrane. (*a*) is a record of the effect of 0·4 mg. tyramine 2 days after removal of the ganglion, (*b*) is a record of the effect of 0·5 mg. tyramine 75 days after removal of the ganglion.

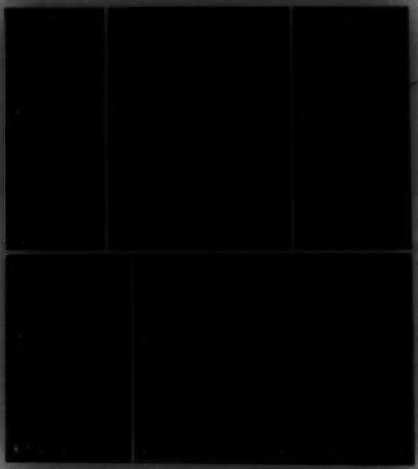


Fig. 3. Records of the denervated (upper) and normal (lower) nictitating membranes from different experiments. In (*a*), (*b*), (*c*), (*d*) and (*e*) the effect of injecting 4·0 mg. tyramine is shown. In (*f*), which is taken from the same experiment as (*e*), the effect of injecting 8·0 mg. tyramine is shown. (*a*) was 2 days after extirpation of the ganglion, (*b*) was 4 days, (*c*) 14 days, (*d*) 21 days, (*e*) and (*f*) 75 days. In (*a*), (*b*), (*c*), (*d*) and (*f*) the contraction of the normal membrane was greater than that of the denervated membrane.

In most of the experiments the larger doses of tyramine caused a greater contraction of the normal membrane than of the denervated.

In Fig. 3 are results obtained by injecting 4 mg. tyramine in cats at different intervals after ganglionectomy. In Fig. 3 (*a*), 2 days after ganglionectomy, the contraction of the denervated membrane is still large, though not so large as that of the normal; in Fig. 3 (*b*), at 4 days' interval, the contraction of the denervated membrane is already small; similarly at (*c*) after 14 days and at (*d*) after 21 days. But in Fig. 3 (*e*) after 75 days the contraction of the denervated membrane is relatively larger; in this cat a contraction in the normal membrane exceeding that in the denervated was obtained only with a dose of 8·0 mg. (Fig. 3 (*f*)). Thus we were unable to discover any progressive change in the response of the denervated membrane to tyramine as time after ganglionectomy lengthened, or to foretell the size of contraction in any experiment.

Contraction of the denervated membrane. The form of the contraction of the denervated membrane in response to tyramine varied greatly as can be seen in Figs. 1, 2 and 3. In some cats the contraction rose slowly to a maximum which was reached later than the maximum in the normal membrane; it also relaxed more slowly (see Fig. 1). In others the contraction began steeply; it then reached a plateau which was maintained longer than the contraction in the normal membrane (Fig. 3 (*d*)). The plateau gave the impression that there was some change in the muscle which made it unable to contract further; when other substances were tested on the same muscle, however, it contracted further, and the form of contraction was very similar to that of the normal membrane.

Summary of the results with tyramine. We performed fifteen experiments in which the response of the two membranes to different doses of tyramine was recorded. In eleven of these the results were as already described, and we have expressed the mean of these results graphically in Fig. 4. The ordinates are the mean heights of contraction of the two membranes, while the abscissæ are the doses of tyramine. For doses below 2·0 mg. the continuous line, representing the response of the denervated membrane, is above the dotted line, representing the response of the normal membrane. For doses above 2·0 mg. the dotted line rises steeply above the continuous line. It should be mentioned that the size of the contraction in the denervated membrane did not depend on the order in which the doses were given. When the large doses were given first the same differences in the contractions of the two membranes were observed.

Unusual results with tyramine. Not less important than the results of these eleven experiments, however, were the results of four others. In two of these the responses of the denervated membrane were very small

to all doses, and the responses of the normal membrane were always greater. In the remaining two experiments the results were quite different, for no matter what dose was used the contraction of the denervated membrane was greater than that of the normal membrane. One experiment was carried out on the day following the removal of the

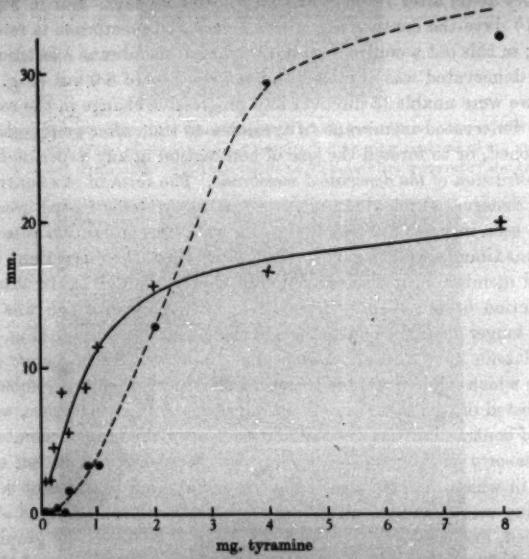


Fig. 4. The curves were drawn by plotting the mean points of eleven experiments in which doses of tyramine were injected. Abscissae are doses of tyramine (mg.), ordinates are heights of contraction of the nictitating membranes (mm.). The continuous line represents the contractions of the denervated membrane and the dotted line the contractions of the normal membrane.

ganglion; in this the greatest difference between the denervated membrane and the normal was seen with the highest dose; there was no suggestion that with still higher doses the normal membrane would contract as much or more than the denervated. The other experiment was carried out 68 days after removal of the ganglion, and although the denervated membrane contracted more than the normal in response to the highest dose injected, it looked as though with a still higher dose the responses might have been reversed. Apart from this surmise, however, the main

point of these two experiments is that the response of the denervated membrane to tyramine was always greater than that of the normal membrane, and the action of tyramine was similar to that which till now we have always supposed to be the action of adrenaline.

II. *Results with other substances*

Dihydroxyphenylethylamine and sympatol. In examining the action of dihydroxyphenylethylamine we have observed results of two kinds. There were results in which the denervated membrane contracted more than the normal in responses to all doses, and there were also results like

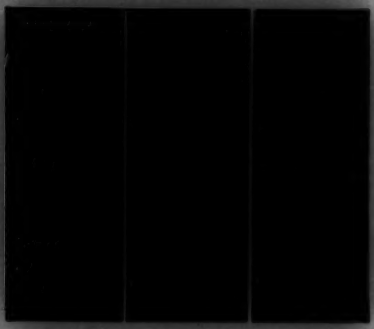


Fig. 5. Contractions of the nictitating membranes to doses of dihydroxyphenylethylamine after excision of the right superior cervical ganglion 33 days previously.

the majority of the tyramine results in which the denervated membrane contracted more than the normal in response to low doses, but contracted less than the normal in response to high doses. An example of this is given in Fig. 5. Out of a total of thirteen experiments, there were five in which the denervated membrane contracted more than the normal for all doses, and six in which the relationship was reversed according to the size of the dose. In the two remaining experiments the effect of large doses was the same in both membranes. The form of the contraction of the denervated membrane was always similar to that of the normal membrane, though the contraction of the denervated membrane was more prolonged.

The number of experiments in which different doses of sympatol were examined was smaller because the effect of this substance on the

nictitating membrane was very prolonged. In six out of nine experiments the denervated membrane contracted more than the normal in response to all doses; in three out of nine the relationship was reversed according to the size of the dose as shown in Fig. 6. In all experiments with these substances the increase of the contraction with increase of dose was steeper in the normal membrane.

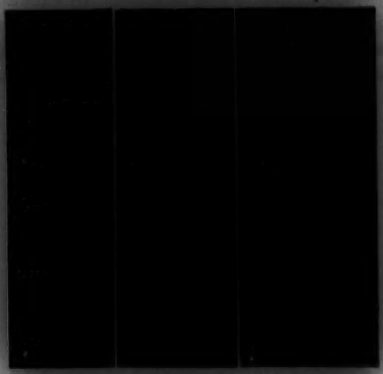


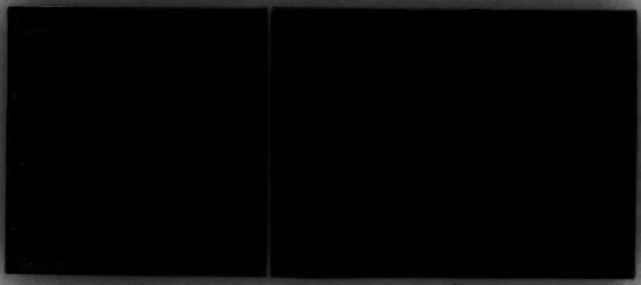
Fig. 6. Contractions of the nictitating membranes to doses of sympatol after excision of the right superior cervical ganglion 21 days previously.

Results with adrenaline. The majority of the results with adrenaline showed, as would be expected, that the denervated membrane contracted more than the normal in response to all doses. Nevertheless, when the mean results of eight experiments were calculated, it was found here also that as the dose increased the contraction of the normal membrane increased more in proportion than did that of the denervated. We would have concluded that this was because the contraction of the denervated membrane was reaching a maximum, but for the surprising observation that in two other experiments, the relative size of the contractions was reversed. One of these experiments was made 1 day after ganglionectomy and the second was made 33 days after ganglionectomy. This second experiment is shown in Fig. 7.

Adrenaline and tyramine infusions. Some experiments were also made in which adrenaline and tyramine were administered by slow intravenous infusion. The results were similar to those given by large single injections, but they brought out more clearly the changed response

in the denervated membrane. In Fig. 8 (*a*) is shown the effect of an adrenaline infusion; the contraction began much earlier in the denervated membrane, but when the normal membrane began to contract it did so more rapidly and the contraction was twice as great. The resemblance of this record to that of the infusion of tyramine in Fig. 8 (*b*)

Fig. 7. Contractions of the nictitating membranes to doses of adrenaline after excision of the right superior cervical ganglion 33 days previously. Note that the normal membrane contracts more than the denervated when the doses are 40 and 100γ.



(*a*) (*b*)

Fig. 8. The effect of slow infusion of adrenaline and of tyramine in a cat 8 days after excision of the right superior ganglion. Both adrenaline and tyramine cause contraction earlier in the denervated membrane, but the contraction is greater in the normal membrane.

indicates the similarity of the effects of adrenaline and tyramine, although in other animals an infusion of adrenaline produced a contraction of the denervated membrane which was throughout greater than that of the normal membrane.

Comparison of the four substances. The actions of the four substances examined can be grouped in three classes; (1) an increased response of the denervated membrane to all doses; (2) an increased response of the denervated membrane to low doses and a decreased response to high doses; (3) a decreased response of the denervated membrane to all doses. In Table I are given the number of experiments with each substance

TABLE I. The figures show the number of experiments in which the indicated response of the denervated membrane was observed

	Increased response to all doses	Increased response to low doses; decreased response to high doses	Decreased response to all doses
Adrenaline	8	2	0
Sympatol	6	3	0
Dioxyphenylethylamine	5	6	0
Tyramine	2	11	2

which fall into each of these classes. The table indicates that in passing from adrenaline to tyramine we pass from a substance most of the responses to which fall in the first class, to a substance most of the responses to which fall in the second class with occasional responses falling in the third class. This transition is supported by a consideration of individual experiments; thus the two experiments in which the response of the denervated membrane was decreased to high doses of adrenaline were the same experiments as those in which the response to all doses of tyramine was decreased. Similarly, the two experiments in which the response of the denervated membrane was increased to all doses of tyramine were experiments in which the response was increased to all doses of other substances. It can be said that in an experiment the results with tyramine are most likely to fall in the second class; the chances are about equal that the results with dihydroxyphenylethylamine will fall in either the first or second class; the chances are in favour of the results with sympatol falling in the first class, and heavily in favour of the results with adrenaline falling in this class. The division of the results into these three classes is certainly justified by the observations themselves, but it is likely to be an artificial classification, which has no real existence. The impression left by the different experiments is that for every substance examined there is a stage after extirpation of the ganglion in which the results would always fall in the first class. As time goes on there is a second stage in which a proportion of the results would fall in the second class; this proportion is low for adrenaline, but becomes greater in passing to sympatol, dioxyphenylethylamine and

tyramine, for which it is high. Finally, there is a third stage in which a proportion of the tyramine results would fall in the third class. Evidence that this transition occurs is entirely circumstantial, for it is impossible to follow it in one animal, and there is so much variation among different animals that even an approximate idea of the duration of each stage cannot be formed.

Ephedrine. An attempt has been made to investigate the behaviour of ephedrine because it is one of the substances which Blaschko *et al.* [1937*b*] have found not to be affected by the enzyme they have described. Ephedrine, however, exerts a very prolonged action on the nictitating membrane, and it is impossible to observe the effect of a series of doses in one experiment unless they are small.

There is no doubt that in response to small doses, 0·3–0·5 mg., the denervated membrane contracts more than the normal membrane. In one experiment this relation was reversed when 4·0 mg. was injected. At the end of another experiment in which a large number of injections had been made, 1·0 mg. ephedrine caused a contraction of the normal membrane but not of the denervated membrane. In a few experiments we have given an initial dose of 4·0 mg. ephedrine, and in these the heights of contraction of the two membranes were very similar. The evidence on the whole indicates that the action of ephedrine is like that of the other substances examined. The one peculiarity of the action of ephedrine, which distinguishes it from the other substances, is that relaxation occurs earlier in the denervated than in the normal membrane.

Extirpation of both ganglia. The possibility occurred to us that some of the phenomena seen in the denervated membrane might be due to incomplete denervation because of postganglionic fibres crossing from the ganglion of the other side. We excluded this by making observations in cats in which both ganglia were removed. The results obtained agreed with those in the other experiments.

Use of anæsthetics. Various workers have used dial as an anæsthetic in making observations on the nictitating membrane. We performed one experiment under urethane, and in this there was no contraction of the denervated nictitating membrane whatever in response to tyramine, though it contracted well to adrenaline. Since we believe that the anæsthetic may influence the results, we have been careful to use the spinal preparation throughout.

Discussion

The evidence indicates that the view previously put forward by Burn is incorrect; there is no qualitative difference between the site of action of tyramine and that of adrenaline; the difference between the action of these substances is quantitative and not qualitative. So far as the nictitating membrane is concerned, a simple conclusion that tyramine acts on the nerve ending while adrenaline acts beyond it is in no way justified. Nevertheless, the reactions of the denervated nictitating membrane to large doses of tyramine fall completely in line with the reactions described by Burn for the pupil and for the vessels of the forelimb; in the majority of animals the contraction of the denervated membrane is much less than that of the normal membrane. But the observation has now been made that in a minority of animals this is also true of adrenaline.

Hitherto there has been little possibility of speculating about the changes which follow denervation; most workers have been content to accept it as a physiological law that denervated tissues are more sensitive to chemical stimuli than normal tissue. The results described indicate, however, that there are at least two changes which are independent of one another.

The one change leads to the increased response of the denervated membrane to low doses; if this change occurs alone it leads to an increased response to all doses. The other change is that which is responsible for the slower rate of increase of contraction of the denervated membrane with increase of dose, illustrated in Figs. 1, 4. Prof. Gaddum has suggested to us that this may be explained by the vascular conditions; that if the vessels are more sensitive after denervation they will be more constricted by bigger doses of tyramine, and the tyramine will not reach the nictitating membrane; therefore, there would be less increase of effect with increase of dose. One of us found, however [Burn, 1932], that tyramine causes less vaso-constriction in a forelimb which is denervated by removal of the stellate ganglion; for this reason we do not think that the suggestion is likely to be correct.

It is possible to explain the increased response of the denervated membrane to low doses in terms of Straub's hypothesis of potential action. Straub considers that for many drugs the intensity of action is proportional to the steepness of the concentration gradient of the stimulating substance across the cell membrane. In a normally innervated tissue it may be that there is always a small amount of the humoral transmitter inside the responsive cell, the presence of which is due to the

continuous liberation of the transmitter by tonic impulses. If the tissue is denervated, this small amount disappears, and if now a drug is injected into the circulation which can act in the same way as the humoral transmitter, it follows that the concentration gradient necessary to cause a visible response will be reached in the denervated tissue before it is reached in the normal tissue; thus denervated tissues are sensitized to the action of drugs in low doses. We doubt, however, whether this explanation is sufficient to explain the increased response of the denervated membrane to high doses which is usually seen when the substance injected is adrenaline. When the dose injected is large, the concentration gradient across the cell membrane must be almost the same on the normal and denervated sides.

The diminished response of the denervated membrane to large doses of tyramine (and occasionally to large doses of adrenaline) is still more puzzling. We have tried to approach this also from the point of view of Straub's theory, since the physiological importance of concentration gradient was impressed upon one of us by the influence of cocaine on the action of adrenaline in the perfused cat's heart. (Burn & Tainter [1931] found that when cocaine was diffusing into or out of the heart, the effect of adrenaline was increased; but when cocaine was present in unchanging concentration, then the action of adrenaline was diminished.) Now in a recent communication [1937] Straub has emphasized that a drug can act efficiently only if there is a mechanism inside the cell for destroying it, for if the drug accumulates the concentration gradient between the outside and the inside of the cell becomes less steep and therefore the cell fails to respond to the drug. The slow removal of the drug would explain, for example, the failure of a second dose of acetylcholine applied to the motor end-plate of the lizard to cause a contraction of the muscle as described recently by Buchthal & Lindhard [1937].

Now Blaschko *et al.* [1937 *a*, *b*] have shown that there is an enzyme system in the tissues which destroys adrenaline and other similar substances. If it is assumed that this system often becomes less efficient after denervation, then when a large dose of a substance such as tyramine is injected there will soon be no concentration gradient between the outside and inside of the denervated cell, and, therefore, on Straub's hypothesis the contraction of the denervated cell will be very small. Furthermore, this small contraction will last longer than the larger contraction in the normal membrane; it will persist until the chemical stimulant has diffused from the cell back into the blood stream since there is no other way for its removal. This is what is observed.

The results obtained with ephedrine do not altogether conform to this explanation.

Blaschko *et al.* found that ephedrine is not destroyed by the enzyme system in the tissues, and we have observed that the effect of ephedrine is actually very prolonged. On the other hand, its action is on the whole similar to that of adrenaline and tyramine; this would not be expected if the rate of removal of the drug inside the cell played an important part in deciding its effectiveness.

As already mentioned, ephedrine is peculiar in that relaxation occurs earlier in the denervated membrane. If ephedrine is not destroyed by any enzyme and can be removed only by diffusion from the tissue cells back into the blood, relaxation will occur earlier in the side with the better blood supply. Now when the forelimb is denervated by extirpation of the stellate ganglion, the blood supply of the soft pads of the foot becomes better than on the normal side. If the same vascular change occurs in the nictitating membrane the denervated membrane will have a better blood supply, and therefore the relaxation after ephedrine will occur earlier on that side.

Knowledge of cell structure and of the mode of action of drugs is still too fragmentary to make it likely that any explanation of the phenomena of denervation which agrees with all the facts can be put forward. To be able to discuss these phenomena in terms of any hypothesis, however, seems to us an advance on the previous view that they were the manifestation of some mysterious law.

Summary

When the effect of tyramine on the denervated nictitating membrane is compared with the effect on the normal membrane in the spinal cat, it is usually observed that when the dose is small the denervated membrane contracts more than the normal membrane, but when the dose is large, the normal contracts much more than the denervated. In a few cats the denervated membrane does not respond to any dose of tyramine, and on the other hand in a few cats the denervated membrane always responds more than the normal.

The interesting observation has been made that in some cats the response of the denervated membrane to large doses of adrenaline also is less than that of the normal membrane. Hence it is not an invariable rule that denervation increases the response to adrenaline; this rule is true for lower doses only.

In passing from adrenaline to sympatol, then to dioxyphenylethylamine and finally to tyramine, there is a gradual change in the effect of denervation on the response to large doses; to adrenaline denervation usually increases the response, to tyramine denervation usually decreases the response.

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THE CARBONIC ANHYDRASE INHIBITOR IN SERUM

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An active inhibitor of carbonic anhydrase found in the sera of pig and other mammals, was briefly described in a recent note [Booth, 1936]. Carbonic anhydrase is normally found only in corpuscles, and its absence from the plasma has distinct advantages in the regulation of the blood *p*H *in vivo* [Roughton, 1935]. The function of the inhibitor was thought to be a protective one in that it provides a temporary mechanism for ensuring that any carbonic anhydrase which may be set free into the circulating plasma as a result of corpuscle destruction does not annul these advantages. Much of the force of this suggestion was lost on finding that the inhibitor is not present in the blood of man. However, even though the inhibitory activity of serum may be accidental it was considered of interest to study (*a*) the kinetic relations of the inhibitor to the enzyme, (*b*) its own chemical nature, and (*c*) its distribution.

Methods

The dehydration rates of carbonic acid were determined with the apparatus of Meldrum & Roughton [1933 *a*] in which bicarbonate solution is suddenly mixed with phosphate buffer and the CO_2 evolution is observed manometrically. The hydration rates (CO_2 uptake by buffer) were determined in a similar apparatus, as described by Meldrum & Roughton [1933 *b*], but with certain modifications. The technique is essentially the same as that used for measuring CO_2 output rates except that CO_2 was added to the manometer vessel (or "boat") and the rate of change of pressure due to its uptake by buffer solution observed manometrically. The boat was first evacuated to 0·1 atm.: then 2 ml. CO_2 at 1 atm. were introduced into the 60 ml. gas space, making the final concentration of CO_2 25 p.c. at 0·133 atm.

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To estimate the inhibitory power of a solution four runs were necessary: (1) the control; (2) the same with the addition of enzyme to the phosphate buffer; (3) with enzyme and test solution; (4) with test solution but without enzyme. The fourth run was made to determine whether the test solution itself contained enzyme. In the case of CO_2 output the accelerations were calculated from the reciprocals of the times taken for the second quarter of the process [Meldrum & Roughton, 1933*a*]. In the case of CO_2 uptake the curves are diphasic, the initial rapid phase being due to physical solution of CO_2. The rates were expressed as the slopes of the second phases. If R_0, R_1, R_2 represent the control, enzyme and inhibited enzymic rates respectively, the enzymic acceleration A_1 in absence of inhibitor is $(R_1-R_0)/R_0$ and in presence of the inhibitor is $A_2=(R_2-R_0)/R_0$. The percentage inhibition is then $(A_1-A_2)\times 100/A_1$.

In CO_2 evolution experiments an inhibitor effect is to some extent simulated by alkali. Hence all test solutions were adjusted to *p*H 7·4. In doubtful cases the genuineness of the inhibitory effect could be checked by making use of the following three facts: (1) alkali decreases the total CO_2 evolved; (2) alkali slows the control rates; (3) alkali accelerates CO_2 uptake (enzymic) while the serum inhibitor slows it. The converse holds throughout in the CO_2 uptake experiments.

Material

Enzyme solutions were prepared from washed pig or horse corpuscles by Meldrum & Roughton's [1933*a*] chloroform method. For certain experiments the solution obtained by laking corpuscles with distilled water was used as source of enzyme without further purification. Serum was used as fresh as possible. When dilution was necessary saline was added.

Kinetics

Inhibitor concentration curves. To constant quantities of pig enzyme solution graded amounts of pig serum were added and made up to a standard volume. Aliquot portions of each mixture were added to the phosphate in the boat and tested (see Fig. 1). In absence of serum the quantity of enzyme used in these experiments accelerated dehydration 3·2-fold. A similar series of curves was also obtained for horse enzyme (chloroform preparation) and pig serum. From the former curves the percentage inhibition was calculated, and Fig. 2 shows how the degree of inhibition depends on the concentration of serum. The enzyme was inhibited almost 90 p.c. by 0·05 ml. serum (with 4·95 ml. total fluid in

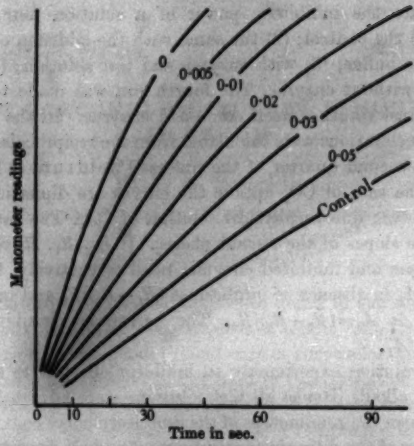

Fig. 1. Inhibition of carbonic anhydrase by pig serum. CO_2 output. 19°. 4 ml. 0·2 *M* phosphate *p*H 7·4 + 0·25 ml. 0·2 *M* bicarbonate + 0·1 ml. enzyme solution; ml. serum as shown by figures on the curves; saline to make total volume up to 4·95 ml. The control without enzyme was also the same when 0·05 ml. serum was added. Maximum acceleration without serum 3·2.

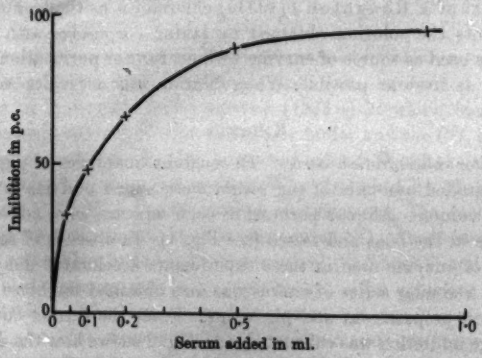

Fig. 2. Percentage inhibition curve. Enzyme constant; serum varied. 19°. Plotted from Fig. 1.

the boat, making the final dilution of serum 1 in 100); and 50 p.c. by 1 in 500. The control curve in Fig. 1, representing the uncatalysed rate, is the same whether serum is added or not. In fact no case of slowing of the control velocity has yet been found. Serum therefore affects the enzyme rather than the chemical reaction itself.

Graded amounts of enzyme were tested with and without serum, the concentration of serum being maintained constant at a final dilution of 1 in 500 (see Fig. 3). The stronger the enzyme solution the lower is the percentage inhibition due to a given amount of serum. Since the range of activity over which the apparatus gives accurate results is narrow the curve is necessarily limited, the value for the percentage inhibition for the highest enzyme activity being least accurate.

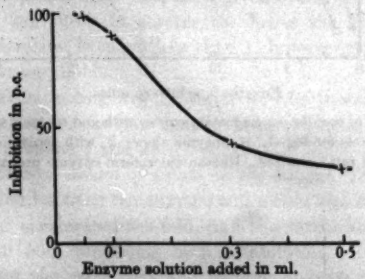

Fig. 3. Percentage inhibition curve. Serum constant; enzyme varied. CO_2 output. 19°. Quantities as for Fig. 1, with 0·1 ml. serum. 0·1 ml. enzyme without serum accelerated 1·6.

Pig serum sometimes contains demonstrable amounts of enzyme. Since the activity of the enzyme must be partly suppressed by the inhibitor normally present, such serum must actually contain more enzyme than is indicated. The activity of any further enzyme added is also found to be partly suppressed, as would be expected from a consideration of Fig. 3. The serum used in the above experiments, although it showed no catalytic activity, may have contained small amounts of enzyme. The true inhibition curve (Fig. 2) would therefore more nearly resemble a rectangular hyperbola. Similar considerations apply to Fig. 3. There would be more enzyme present when serum was added than in its absence: hence the percentage inhibition must have been greater than was observed.

A series of runs was made with graded amounts of a chloroform enzyme preparation (human) when the concentration curve *A* in Fig. 4

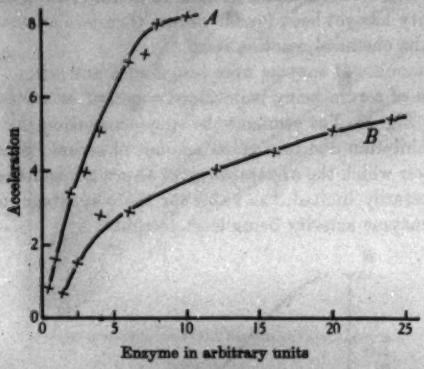

Fig. 4. Comparison of enzyme concentration curves with and without serum. CO_2 output. 14°. Quantities as for Fig. 1. *A*, enzyme alone; *B*, with serum: enzyme and serum both varied but ratio constant. Human chloroform enzyme preparation. Pig serum.

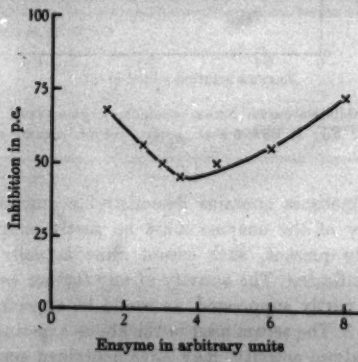

Fig. 5. Percentage inhibition curve. 14°. Enzyme and serum varied together. Plotted from Fig. 4.

was obtained. To this same enzyme solution pig serum was added in such amount as to halve a normal fourfold inhibition, 1 vol. serum being required for 5 vol. enzyme solution. A series of runs was made using

from 0·04 to 0·6 ml. of this enzyme-serum mixture. The concentration curve obtained is shown for comparison in Fig. 4. One essential difference is that the acceleration in absence of serum reaches a maximum of 8·2 and in presence of serum of only 6. The diffusion rates from liquid to gas phase in the apparatus probably set these limits to the observed accelerations. Serum proteins may interfere with this diffusion. At any rate, certain other proteins lower this maximum acceleration, although they show no inhibitor effect at moderate enzyme concentrations. From these two enzyme concentration curves (with and without inhibitor) the percentage inhibition for constant enzyme/serum ratio has been calculated and plotted in Fig. 5. The minimum inhibition was obtained with an enzyme concentration which accelerated (in absence of inhibitor) 4·5-fold. Again it becomes clear that for a given concentration of inhibitor the percentage inhibition is greater the lower the enzyme strength. When the acceleration is much less than 1, however, the experimental method becomes unreliable.

Therefore when testing any material for the presence of inhibitor the enzyme concentration has usually been adjusted to produce an acceleration of between 1 and 2½.

Effect of prolonged action of serum on the enzyme. The effect of prolonged interaction between the enzyme and serum was investigated. The enzyme solution accelerated 3·2-fold, and the serum added had a final dilution of 1 in 500, producing 50 p.c. inhibition. Curves *E* and *A* (Fig. 6) represent the rates with and without enzyme respectively. An enzyme-serum mixture was tested at once and again after 4½ and 7 hr. respectively. The several curves (*B*) coincided exactly, showing that the phenomenon is a genuine inhibition and not due to destruction of enzyme. The same amounts of enzyme and of serum were also added directly to the buffer in the boat, without previous mixing, and tested after the shortest practicable equilibration period. The rate, as shown by curve *C*, is minutely faster than rate *B*. For *D* the enzyme was added to the buffer and the serum to the bicarbonate: thus inhibitor and substrate came into contact with the enzyme simultaneously. The rate here was a little greater initially but after 20 sec. was the same as for *B*.

Inhibition of hydration. Fig. 7 shows the effect of serum on CO_2 uptake by phosphate buffer *p*H 7·4. The amount of enzyme solution used accelerated CO_2 uptake 2·6-fold. When 0·02 ml. serum was added (total fluid 4·20 ml.) the acceleration fell to 0·26-fold—that is, an inhibition of 90 p.c. was obtained by 1 in 210 serum. This is in reasonable agreement with the values for the inhibition of CO_2 output.

Effect of phosphate concentration. The CO_2 uptake method at low pressure allows variation of the concentration of the phosphate buffer. In experiments carried out with Dr F. J. W. Roughton the control rate was found to increase with the phosphate concentration. Later I tested the effect of serum on the enzyme at two phosphate concentrations at 17°

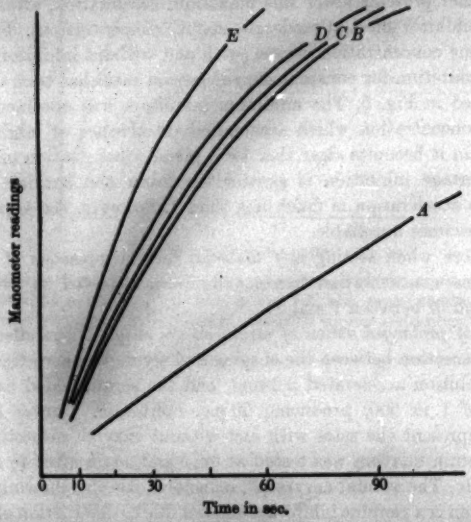

Fig. 6. Effect of prolonged contact between enzyme and serum. CO_2 output. 18°. Quantities as for Fig. 1. *A*, control without enzyme; *E*, enzyme without serum; *B*, *C*, *D*, same amount of enzyme as for *E* but with 0·01 ml. serum added in various ways: see text for details.

and *p*H 7·3. Pig serum (1 in 110) depressed the enzyme-accelerated rates with 0·2 *M* and 0·02 *M* phosphate by 85 and 75 p.c. respectively. Serum had no effect on the control rate at either phosphate concentration. Most of the experiments in this paper (whether CO_2 output or uptake) were done with about *M*/6 phosphate: the concentration was never varied within one experiment.

Effect of temperature. Temperature had an unexpected effect on the efficacy of serum as inhibitor. The experimental results are summarized

in Table I. It is clear that a smaller amount of serum has a greater inhibiting effect on a larger amount of enzyme at 36° than at 0°.

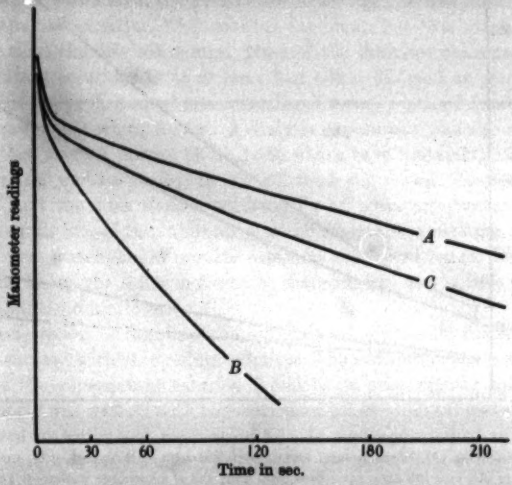

Fig. 7. Inhibition of carbonic anhydrase by serum. CO_2 uptake. 0°. 4 ml. 0·2 *M* phosphate *p*H 7·4 + 0·2 ml. enzyme, serum and/or saline. 25 p.c. CO_2 in 0·133 atm. total pressure. *A*, control; *B*, with enzyme; *C*, with enzyme + 0·02 ml. serum.

TABLE I. Effect of temperature on serum inhibitor

CO_2 uptake. 25 p.c. CO_2 in total pressure of 0·133 atm. 5 ml. 0·023 *M* phosphate *p*H 7·3. Pig serum and 1 in 200 pig chloroform enzyme solution as shown. Water to 5·5 ml.

Temperature °C.	Enzyme solution ml.	Serum ml.	Inhibition p.c.
0	0·06	0·05	65
17	0·12	0·05	80
36	0·4	0·02	80

Effect of p*H*. Fig. 8 shows the *p*H activity curves with and without enzyme, and with enzyme and serum both present. The percentage inhibition by 1 in 1340 serum (calculated from *C*, *B* and *A*) is plotted in Fig. 9. Although the activity of the enzyme increases with rise in *p*H, the increase in the effectiveness of the serum inhibitor is even greater, as shown by the rise in the p.c. inhibition curve. In another experiment using three times as much serum the inhibition was approximately constant at 90 p.c. irrespective of *p*H.

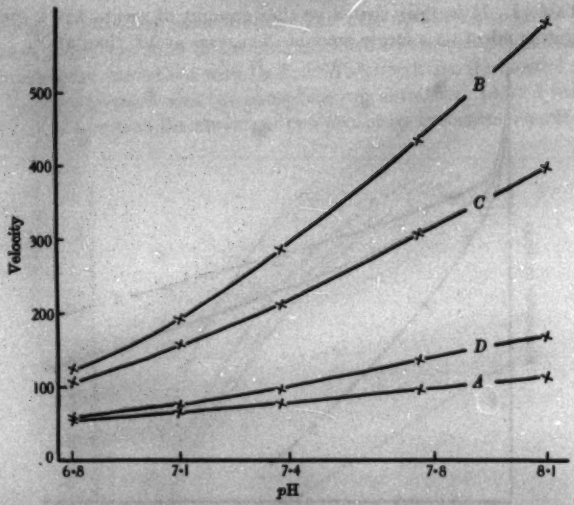

Fig. 8. Effect of pH. Enzyme and serum both constant; pH varied. CO_2 uptake. 0°. 25 p.c. CO_2 in 0·133 atm. total pressure. 4 ml. 0·2 *M* phosphate buffer + 0·2 ml. water or other solution. Pig chloroform enzyme preparation. Pig serum. *A*, control without enzyme; *B*, with enzyme; *C*, with enzyme + serum 1 in 1340; *D*, with enzyme + serum 1 in 420.

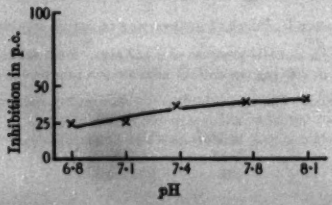

Fig. 9. Percentage inhibition curve. Enzyme and serum (1 in 1340) both constant; pH varied. Plotted from Fig. 8.

The nature of the inhibitor

The chemical nature of the inhibitor was studied by fractionating serum and testing each fraction on a horse enzyme solution.

Dialysis. Fresh pig serum was dialysed for 48 hr. in cellophane tubes, first against running tap water, then against two changes of distilled

water. The temperature averaged 10°. The volume increased by about 50 p.c. and a precipitate of euglobulin formed. An equal volume of distilled water was added, the precipitate centrifuged off and dissolved in a small amount of saline. This solution inhibited, but to a much smaller extent than the original serum. Most of the inhibitor remained in the supernatant solution: little or none had either dialysed or precipitated out. This dialysed, diluted and centrifuged serum was used for several of the experiments which follow. A dialysis experiment was also arranged in another way. A narrow (1 cm.) cellophane tube containing 10 ml. distilled water was suspended in 500 ml. fresh pig serum. The serum was stirred and the tube shaken occasionally at room temperature over a period of 48 hr. If the inhibitor is at all dialysable this large excess of serum over water should provide excellent conditions for its removal by dialysis. When the solution from the dialysing sac was tested, however, it contained no inhibitor.

Precipitation. A sample of diluted, dialysed, euglobulin-free serum was half saturated with ammonium sulphate. The precipitate was centrifuged off and the supernatant solution found to be only slightly active. The precipitate was washed with half-saturated ammonium sulphate solution, dissolved in water and reprecipitated. An aqueous solution of this last precipitate was very active. Euglobulin, obtained from fresh serum by precipitating by one-third saturation with ammonium sulphate, was found to inhibit only to a small extent. On the other hand the washed and neutralized precipitates obtained by adding one-third of its volume of 5 p.c. hydrochloric acid to serum, or by saturating with magnesium sulphate, were very active. Thus most, but not quite all, of the inhibitor followed the pseudoglobulin fraction. Neither magnesium nor ammonium sulphates or chlorides themselves inhibited carbonic anhydrase.

Denaturation. Fresh serum was boiled at *p*H 5·4 for 3 min. The coagulum was filtered off and suspended in saline. Both the filtrate and the suspension were adjusted to *p*H 7·4 and tested. Neither showed any inhibition. To a portion of the dialysed, diluted and centrifuged serum from above an equal volume of water was added (corresponding to serum diluted 1 in 5) and a few drops of alkali. This solution was boiled for 4 min. to convert the protein to metaprotein. No precipitate formed but the solution became opalescent, particularly on cooling and adjusting to *p*H 7·4 for testing. It was moderately active.

Other protein denaturing processes were also applied to serum. 1 vol. cooled dialysed serum was added to 2 vol. cooled ethyl alcohol and allowed to stand at 2° for 1 hr. This treatment completely and irreversibly

precipitates all the protein, which can be filtered off leaving an a-biuret filtrate. The precipitate, which was dried, suspended in saline and tested at once, was inactive. The filtrate was also inactive after the alcohol had been distilled off *in vacuo*. The experiment was repeated with acetone. The a-biuret filtrate was inactive. The precipitate inhibited if dissolved and tested at once but not if allowed to dry. Acetone itself, used as a control, did not affect the enzyme. Alcohol denatures serum proteins and converts them to an insoluble form resembling a heat coagulum. Acetone also denatures and completely precipitates the proteins but the precipitate only becomes insoluble on drying.

Association with protein. These experiments are summarized in Table II. The inhibitions have not been expressed as percentages and are

TABLE II. Solubility of the inhibitor. Serum fractions tested on enzyme from horse blood

Treatment applied to serum		Ppt. dissolved or suspended in salt solution pH 7·4	Filtrate or centrifugate
1. Dialysis and dilution	s	+	+ + + +
2. Dialysate	.	.	0
3. Acidified with HCl	s	+ + +	+ +
4. 1/3 saturated $(NH_4)_2SO_4$	s	+	+ + + +
5. 1/2 saturated $(NH_4)_2SO_4$	f	+ + + +	+
6. 1/2 saturated $(NH_4)_2SO_4$	d	+ + + +	+
7. Saturated $MgSO_4$	d	+ + + +	+
8. Boiled at pH 5·4	s	0	0
9. Boiled at pH >8	d	No ppt.	+
10. Added alcohol at 2°	d	0	0
11. Added acetone at 2°	d	.	0
Ppt. not dried	.	+ +	.
Ppt. dried	.	0	.
12. Controls: $(NH_4)_2SO_4$	.	.	0
$MgSO_4$	.	.	0
NaCl	.	.	0
Acetone	.	.	0

s = fresh serum. + = inhibitor present.
d = dialysed serum. 0 = inhibitor absent.
f = solution from Exp. 4.

not all strictly comparable. Nevertheless certain conclusions can be drawn as to the nature of the inhibitor.

(1) It is not dialysable.

(2) Exps. 7–10 indicate that its activity is bound up with the solubility of the serum proteins. When the proteins are rendered less soluble as by converting to metaprotein, or insoluble as by coagulating, the inhibitor is respectively weakened or destroyed.

(3) It is always precipitated with the protein. Much the greatest proportion follows the pseudoglobulin, while the euglobulin and albumin fractions (or at least the fraction precipitated by one-third, and the

fraction not precipitated by one-half, saturation with ammonium sulphate) show comparatively little activity. There is some evidence [Chick, 1914; Rimington, 1933] that euglobulin is (wholly or partially) denatured pseudoglobulin: in that case the decrease in activity accompanying denaturation is in agreement with the lower activity of euglobulin. It is possible that some protein closely resembling pseudoglobulin may not be completely precipitated by half saturation with ammonium sulphate and that this accounts for the activity of the "albumin" fraction.

Although the evidence for the protein nature of the inhibitor is not complete there can be little doubt that it is closely associated, if not identical, with serum globulin.

Is the inhibitor an artifact?

For most of the work on the inhibitor a "crude chloroform preparation" was kept in the refrigerator and used over a period of several days, whereas the serum was generally used fresh. Enzyme solutions prepared by laking corpuscles from various sources with distilled water were also inhibited by sera from several animals. Pig serum frequently contains visible amounts of hæmoglobin, and presumably therefore carbonic anhydrase, yet no enzyme activity can be demonstrated. This can only mean that the serum inhibitor completely suppresses enzyme activity at this crude stage of purity as well as at the more advanced stages. In more than one experiment an enzyme preparation from pig blood was found to be inhibited by serum from the *same specimen* of blood: hence there can be no question of a "foreign protein" effect.

Since serum differs from plasma the results were checked with plasmas obtained by mixing freshly drawn blood with (*a*) heparin, and (*b*) potassium oxalate, and centrifuging. Both plasmas inhibited to the same extent as serum. Oxalate itself had no effect on the enzyme. It is clear that the inhibitor is not formed during the production of serum. Indeed there is no reason to disbelieve that the inhibitor exists in the blood *in vivo*.

Distribution

Mammalian sera. The sera or treated plasmas from various mammals and from birds have been tested (see Table III). While the inhibitor is found in the sera of several domesticated mammals, it is noteworthy that human blood contains none. Nor is any found in the sera from monkey, duck, or pigeon. The tests on the distribution of the inhibitor were performed at room temperature. Because the activity of pig serum is greater

TABLE III. Distribution of the inhibitor

Material	Carbonic anhydrase	Enzyme preparation used	Inhibitor
Pig serum	0	h	+ + + + + +
Pig serum	.	m	+ + + + + +
Pig serum	.	p	+ + + + + +
Pig heparinized plasma	0	p	+ + + + + +
Pig oxalated plasma	.	p	+ + + + + +
Pig serum and oxalate	.	p	+ + + + + +
Human serum	0	p	0
Human serum	.	m	0
Human heparinized plasma	.	m	0
Human oxalated plasma	0	p	0
Human oxalated plasma	.	m	0
Human jaundiced serum (1)	.	p	0
Monkey serum (2)	0	p	0
Ox serum	0	p	+ +
Horse serum (heated)	.	p	+ +
Cat oxalated plasma	.	p	+ + + + +
Rat serum	.	p	+ + + + + + +
Rat serum	.	m	+ + + + +
Sheep serum	.	p	+ + +
Sheep fœtal serum (85 day) (3)	.	m	+ + +
Rabbit maternal oxalated plasma	Trace	r	+
Rabbit fœtal oxalated plasma	Trace	e	+
Guinea-pig serum	.	p	+
Fowl whole plasma (5)	0	p	Trace
Fowl whole plasma (5)	.	f	Trace
Fowl serum (5)	.	f	Trace
Duck serum (4)	0	m	0
Pigeon serum	0	ox	0
Human urine	A	p	0
Cat urine	0	ox	0
Cow's milk	A	p	0
Pig bile	Trace	p	0
Human saliva	.	p	0
Pig stromata	Trace	p	+
Witte's peptone	.	p	+
Globulin from cow's whey	.	p	Trace
Snail blood (*Helix pomatia*) (6)	0	p	0
Maia blood (6)	0	p	0
Hamlet serum (7)	Trace	p	Trace
Octopus blood (7)	.	p	0
Commercial blood albumin	.	h	+
Commercial egg albumin	.	p	0
Fresh egg-white	.	p	Trace
Glaucoma pyriformis, whole (1)	0	.	—
Glaucoma pyriformis, disintegrated (1)	0	p	Trace
Baker's yeast suspension	A	p	0
Potassium oxalate (as control)	.	p	0

Explanation of signs

Material (1) supplied by Dr N. W. Lawrie, (2) by Prof. E. D. Adrian, (3) by Sir Joseph Barcroft, (4) by Dr C. Ludwig, (5) by Mr E. N. Willmer, (6) by Dr E. Baldwin, (7) by Dr F. J. W. Roughton.

\+ = inhibitor (or enzyme) present.
0 = inhibitor (or enzyme) absent.
A = enzyme shown absent by Meldrum & Roughton [1933a].
h = horse chloroform enzyme preparation.
m = human chloroform enzyme preparation.
ox = ox chloroform enzyme preparation.
p = pig chloroform enzyme preparation.
f = fowl laked corpuscle solution.
r = rabbit laked corpuscle solution.
e = rabbit fœtus laked corpuscle solution.

at 36° than at 17° human serum was also tested at 37°. No inhibition was observed.

Meldrum & Roughton [1933*a*] compared the concentration of enzyme in fœtal and maternal goat blood. They found the former was weak until about 4/5 full term when it rapidly approached the maternal value. It was of interest to know whether the inhibitor makes its appearance simultaneously. One specimen of fœtal sheep serum was obtained from an 85-day fœtus. This is about 3/5 full term. The fœtal serum showed about the same activity as that from an adult. Rabbit serum inhibited only slightly, but here too the fœtal serum was as effective as the maternal.

Miscellaneous biological material. Certain other physiological fluids have been tested. The results appear in Table III. Milk, urine, and bile from animals whose sera are active contain no inhibitor. Bloods from certain lower animals which contain neither corpuscles nor carbonic anhydrase also contain no inhibitor. Various preparations from mammalian tissues were tested. Globulin was precipitated from cow's whey by half saturating with ammonium sulphate. This material contains a powerful xanthine oxidase which inhibits certain enzymes, e.g. amino acid oxidase [Keilin & Hartree, 1936], and glucose dehydrogenase [Harrison, 1933]; it also contains nucleosidases which destroy cozymase [Booth, unpublished]. The inhibiting effect of this globulin on carbonic anhydrase was feeble. Stromata were prepared from pig corpuscles, washed twenty times and suspended in saline. The preparation has some slight enzyme activity and a small though definite inhibitory effect.

Two samples of *Glaucoma pyriformis* were tested. One was a suspension of the living protozoon and the other had been disintegrated by repeated freezing and thawing. Neither contained any carbonic anhydrase but the latter inhibited pig enzyme slightly.

When a trace of enzyme was found in any preparation this was frequently accompanied by measurable amounts of the inhibitor. Although the latter does not completely inhibit the enzyme already present it partially inhibits any extra enzyme which is added. This agrees with the finding in the section on kinetics, that percentage inhibition varies with the strength of enzyme.

In mammals the inhibitor is probably confined to the blood (and lymph) although a definitive conclusion must await further results. Certain other biological materials show barely measurable inhibitory effects: this, however, may be due to physical rather than chemical causes.

Discussion

The fact that the inhibitor is absent from the bloods of certain animals (man and monkey in particular) weakens the force of the original suggestion that its function is a protective one. However, it is of interest to evaluate the possible extent of this protection in the case of the pig. At 17° 0·05 ml. serum inhibits 0·1 ml. of 1 in 200 laked blood 90 p.c. and the inhibitor is nine times as effective at 37°. The serum from 100 ml. pig blood (amounting to 55 ml.) would inhibit (to 90 p.c.) the enzyme from $\frac{55}{0{\cdot}05} \times \frac{0{\cdot}1}{200} \times 9 = 4{\cdot}95$ ml. blood. That is, the inhibitor in circulating blood could only effectively inhibit the enzyme liberated by lysis of at most 5 p.c. of the corpuscles. We may reasonably assume a mean life of 25 days for corpuscles. A less certain but not unreasonable assumption is that not more than one-quarter of those undergoing destruction do so by fragmentation in the blood. About 1 p.c. of the total enzyme would then be liberated into the plasma per day. The inhibitor could suppress 90 p.c. of 5 days' accumulation.

The experiments described in this paper prove that the inhibitor can function *in vitro* but we know nothing of its effect *in vivo*. Even if the inhibitor does perform the suggested function it can at most only be a temporary one and in a limited number of species. The mechanism of the final disposal of the enzyme from the tissues remains unknown.

Summary

1. An active inhibitor of carbonic anhydrase has been found in the sera from pig, sheep, horse, ox, cat, and rat, in oxalated and heparinized plasmas and in fœtal sera.

2. The inhibitor is absent from the bloods of man, monkey, duck and pigeon.

3. The inhibitor is not found in milk, urine, or bile from animals whose sera inhibit, nor in blood (of certain lower animals) which contains no carbonic anhydrase.

4. The inhibitor has many of the properties of pseudo-globulin from which it has not been separated.

5. Serum has no effect on the uncatalysed rates of CO_2 uptake or output but inhibits the enzymic acceleration of both to about the same degree.

6. Percentage inhibition increases with serum concentration, and for a given amount of serum, the inhibition decreases with increasing amount

of enzyme. The inhibition is not increased by prolonged contact between enzyme and serum and hence is not due to destruction of enzyme.

7. The inhibition increases with rise in temperature but is only slightly affected by *p*H.

8. The inhibitor in pig blood could suppress 90 p.c. of the enzyme liberated from about 5 p.c. of the corpuscles.

9. The originally suggested protective function is discussed in the light of the absence of the inhibitor from human blood.

I wish to thank Dr F. J. W. Roughton for helpful criticism and advice during the course of the latter part of this work and for the use of his apparatus.

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16 *October* 1937

The intrinsic gastro-intestinal fibres are connecting fibres.

By P. Nolf. (*Brussels*)

If, in a fowl whose spinal cord is destroyed and whose vagus nerves are cut, a duodeno-ileal loop, including the greater part of the duodenum and the first 5 or 6 cm. of the ileum, and a loop about 12 cm. long, following immediately on the preceding and consisting of the upper part of the ileum, are isolated, it is usual for the first loop to have regular contractions with the same rhythm as the gizzard. Exceptionally, the same rhythm may be observed in the second loop. Given that these loops are separated from each other by a complete transversal section of the intestinal wall and that the first loop and the gizzard are similarly separated from each other, isorhythm implies the existence of gastro-intestinal fibres arising from the intrinsic nervous system of the gizzard and distributed to the different segments of the small intestine along a track lying outside the intestinal wall.

These intrinsic gastro-intestinal fibres, just like the extrinsic gastro-intestinal nerves, can exercise either a motor or an inhibitory effect. In the first alternative, each one of the rhythmic contractions of the gizzard is accompanied by a contraction of the intestinal loop. In the second, it either causes a transitory relaxation or stops the spontaneous contractions of the loop, or does both simultaneously. The motor effect may be seen in the loops, the wall of which is relaxed between two successive contractions arising in the gizzard; the inhibitory effect occurs in those whose wall, for some reason or other, is in a state of permanent contracture.

If, through direct faradic stimulation of the nervous apparatus of the gizzard, the impulses of the latter become more intense and more frequent, it may happen that a loop originally relaxed progressively enters into a state of contracture which may last several minutes. All this

while, the loop no longer responds to each contraction of the gizzard by an isorhythmic contraction but by an isorhythmic relaxation. Its reaction is inverted.

Sometimes the two loops enter into contracture successively, contracture in the one coinciding with relaxation in the other. The loop which is in contracture responds by relaxation, the other by contraction, to the self-same contraction of the gizzard, and thus each alternatively displays the inversion phenomenon.

These facts, and the paralysing action of nicotine on the gastro-intestinal fibres, are best explained by admitting that the latter are not themselves either motor or inhibitory fibres, but that they exercise their influence through the motor and inhibitory neurones contained in the wall of the gut. They belong to the system of connecting fibres previously described in the gastro-intestinal plexus of the bird.

Liberation of histamine from the perfused lung of the guinea-pig by bee venom. By W. Feldberg and C. H. Kellaway

Recently we have shown that snake venoms liberate histamine from perfused lungs. The action of bee venom resembles that of snake venoms in many respects. We have therefore applied to bee venom the method we used for demonstrating the liberation of histamine by snake venoms.

A solution of bee venom containing the equivalent of 50 stings per c.c. in a clear glycerine water extract was specially prepared for us by Mr David G. Bull. The solution contained no recognizable quantity of histamine, certainly less than 0·2γ per c.c. The guinea-pig's lung was perfused from the pulmonary artery with Tyrode solution. The fluid flowing from the left auricle and leaking from the lung surface was collected and assayed for histamine on the isolated intestine of the guinea-pig. Before the injection of venom the outflowing fluid was devoid of activity or had only a slight stimulant action. After the injection of 0·1 c.c. of bee-venom solution it became active. This stimulant activity was clearly caused by the presence of at least two principles readily distinguishable by their action on the gut. One caused a rapid, almost immediate, contraction and the other a delayed and slow contraction. The rapidly contracting substance was identified with histamine (1) by its depressor action on the blood pressure of the atropinized cat, (2) by a quantitative comparison of its gut-contracting and depressor actions, and (3) by the fact that it was part of the lung histamine.

The histamine output increased during the first 60–90 min. after the injection, and then decreased again. Samples collected after 3 and 4 hours still contained histamine. The total output after the injection of five stings corresponded to between 65 and 75 p.c. of the lung histamine. The histamine output in a typical experiment is shown in Fig. 1. The total amount of histamine assayed in the outflowing fluid from the right

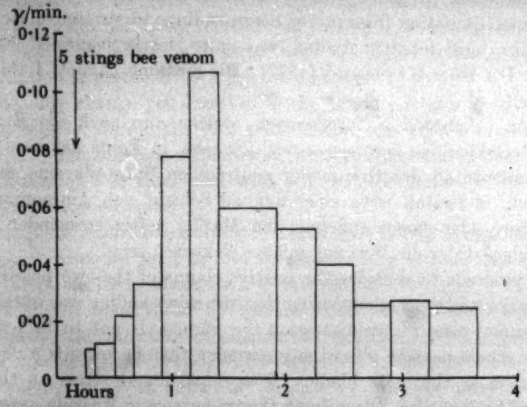

Fig. 1. Histamine output in γ per min. from perfused guinea-pig's lung after bee venom. Ordinates, output in γ per min.; abscissæ, time in hours. The sample collected during the first few minutes was not tested.

lung was 9·13γ; the lung was then extracted and yielded 3·2γ. In another experiment the total output in 3½ hours was 9·04γ and the lung yielded by extraction another 5·1γ. The histamine content of the unperfused left lung was 13γ. The histamine of the perfusate is therefore derived from the lung histamine.

The liberation of histamine demonstrated in our experiments must be taken into consideration in explaining those symptoms of bee venom poisoning which resemble the effects of histamine.

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Du Bois-Reymond and chemical transmission.

By H. H. Dale

It is now generally recognized that the chemical transmission of nervous effects, though studied experimentally only since Loewi's demonstration in 1921, was suggested some 17 years earlier by Elliott to explain the similarity of the effects of adrenaline to those of sympathetic nerve impulses. It recently came to my notice that a chemical transmission of excitation from motor nerve endings to striated muscle, the possibility most recently studied, was quite clearly suggested 60 years ago by Du Bois-Reymond [1877]. His meaning cannot, I think, be mistaken.

"Von bekannten Naturprocessen, welche nun noch die Erregung vermitteln könnten, kommen, soviel ich sehe, in Frage nur zwei. Entweder müsste an der Grenze der contractilen Substanz eine reizende Secretion, in Gestalt etwa einer dünnen Schicht von Ammoniak oder Milchsäure oder einem anderen, den Muskel heftig erregenden Stoffe stattfinden. Oder die Wirkung müsste elektrisch sein."

He proceeds to consider the relative claims of the two possibilities. If Krause was right in supposing that the nerve ending was outside the sarcolemma, electrical transmission, he admits, is not to be avoided; but the whole passage gives the impression that he regards a chemical mechanism as the more likely. He lays particular stress on the fact observed by Brücke, "dass durch Curara entnervte Muskeln gegen kurz dauernde elektrische Ströme unterempfindlich sind", arguing that it is difficult to suppose that the contractile substance would have been endowed with a low sensitiveness to its adequate stimulus.

Though such teleological reasoning makes no strong appeal, there can be no doubt as to Du Bois-Reymond's priority in suggesting a chemical mechanism of transmission—at least until an earlier claimant is discovered.

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The action of potassium chloride on mammalian muscle.

By G. L. Brown

The effects of the close arterial injection of KCl solutions into the normal gastrocnemius and tibialis anticus muscles of the cat have been observed. In concentrations between 5 and 100 mg. per c.c. injected

in volumes of 0·5 c.c. into gastrocnemius and 0·25 c.c. into tibialis anticus, KCl causes an immediate, twitch-like contraction, closely resembling that evoked by acetylcholine in 1000 times smaller concentration. Electrical examination of the muscle, by concentric needle electrodes, amplifier and oscillograph, shows that the potassium contraction is accompanied by a brief outburst of asynchronous, diphasic action potentials, which again closely resemble those produced by acetylcholine. If the injection is interpolated in a regular series of single maximal nerve volleys, 10 sec. apart, it is followed by an increase, up to 45 p.c., in the twitch tension, which may last for some 15 min.; with the higher concentrations, the potentiation is preceded by a brief depression.

The only change yet observed in the action potential (belly tendon) of the muscle, during the KCl potentiation, has been a *diminution* in the E.M.F. Thus, in one experiment, a 25 p.c. increase in the maximal twitch tension was coincident with a 50 p.c. diminution in the accompanying action potential.

Complete curarine paralysis to nerve stimulation leaves unaffected the quick response to KCl, and its potentiating action, then observed with direct electrical stimulation of the muscle.

In muscle denervated by section of its nerve supply some days previously, the injection of similar concentrations of KCl produces a quick contraction, accompanied by oscillatory action potentials, followed by a contracture with electrical "silence"—again like acetylcholine. If the injection is made during a regular series of direct electrical excitations of the muscle, the immediate response is followed by a potentiation, similar in degree and duration to that observed when the muscle is excited through its nerve.

The potentiating action of KCl therefore appears to involve the general reaction of the muscle fibre itself, and not the neuro-muscular transmission exclusively.

A local electric response in crustacean nerve. By A. L. HODGKIN.
(*From the Department of Physiology, Cambridge*)

Katz [1937] has recently described experiments which suggest that a subthreshold stimulus sets up a local electric response. In crustacean nerve the existence of this response can be proved by recording the electrical changes produced by brief induction shocks. The experiments described in this communication were made with single fibres from the

limb nerves of *Carcinus Maenas* (the Shore Crab). The nerve trunk was dissected under sea water, and one of the largest axons (about 30μ in

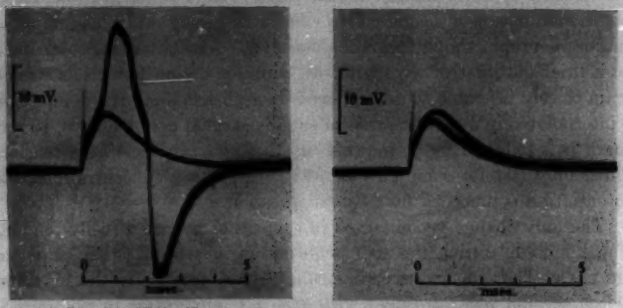

Fig. 1. Electrical changes produced by brief threshold shocks (duration, <0·1 msec.); recorded from cathode. Several responses are superimposed in each record.

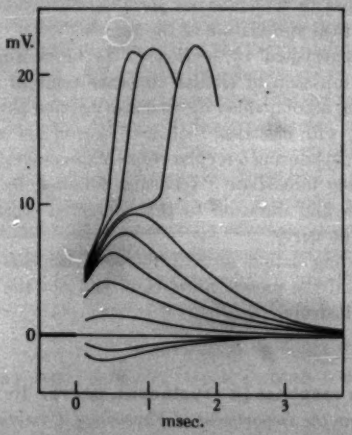

Fig. 2. Electrical changes produced by shocks with relative strengths (successively from above) 1·02, 1·00, 1·00, 1·00, 1·00, 1·00, 0·95, 0·57, −0·57, −1·00.

diameter) attached to fine platinum hook electrodes. The fibre was insulated for electrical recording by raising it into a layer of paraffin oil, which floated above the sea water containing the dissected nerve. The

manipulation involved in the isolation of a single fibre does not seem to affect its activity, for axons prepared in this way can transmit action potentials of 10–30 mV. at a frequency of about 300 per min. for an hour or more. The propagated action potential is of constant size and form, but it is always preceded by a local response whose amplitude and duration vary with the strength of the stimulus. This was observed by connecting the cathode of the stimulating circuit to the ground lead of the amplifier. The records in Fig. 1 were made in this way; they show that the response to a threshold shock alternates between a small monophasic wave and a large diphasic action potential. The shape of the monophasic wave suggests that it is a local response of the stimulated region, and this conclusion is strengthened by the fact that it is abolished in the absolute refractory period.

The development of the local response is illustrated by the experimental curves in Fig. 2. With a stimulus of 0·57 threshold there is a small electrical change, which must be partly due to the polarization produced in the nerve and in the common stimulating and recording electrode. There is also a suggestion of local activity, for the effect of a cathodal shock is greater than that of an anodal one. At 0·95 threshold the local response is quite distinct and makes a hump on the polarization curve. When the stimulus is brought into the range of the fluctuating threshold, the response is considerably increased, and its amplitude now varies from shock to shock. The transition from local to propagated response occurs when the depolarization exceeds a critical value. If the response fails to reach this value, it does not propagate and dies out as a local monophasic wave.

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Pro-gonadotropic sera. By I. W. Rowlands.
(*From the National Institute for Medical Research, Hampstead*)

In the course of an investigation into the specificity of anti-gonadotropic sera [Rowlands, 1937] certain instances were found in which the prolonged injection of gonadotropic extracts led to the serum of the recipient acquiring the power to augment the gonadotropic activity of certain extracts on immature rats:

(1) The serum of a sheep injected daily for 3½ months with extract of sheep pituitary failed to show anti-gonadotropic action against any

extract, but had the power to augment the gonadotropic activity, on rats, of extract of sheep pituitary.

(2) The serum of a goat similarly injected with extract of pig pituitary, inhibited the gonadotropic activity of extracts of horse pituitary, pregnant mare serum and human urine of pregnancy, but at the same time augmented the gonadotropic activity, on rats, of extracts of sheep, pig and ox pituitary.

(3) Antisera to ox and horse pituitary extracts, prepared in rabbits, were inhibitory to nearly all gonadotropic extracts, but were augmentatory to those of pig pituitary.

No augmentation of the capacity of any of the extracts to cause ovulation in the œstrous rabbit was produced by the sera.

Control experiments on rats showed that normal sera gave only slight augmentation, which was probably due to alteration of conditions of absorption. In view of the specificity of the augmenting effect, and of the somewhat similar results obtained by Collip [1937] and Thompson [1937], it must be concluded that the action is of significance. It is possibly due, as Thompson suggests, to the production of substances which neutralize the pituitary antagonist described by Evans *et al.* [1936].

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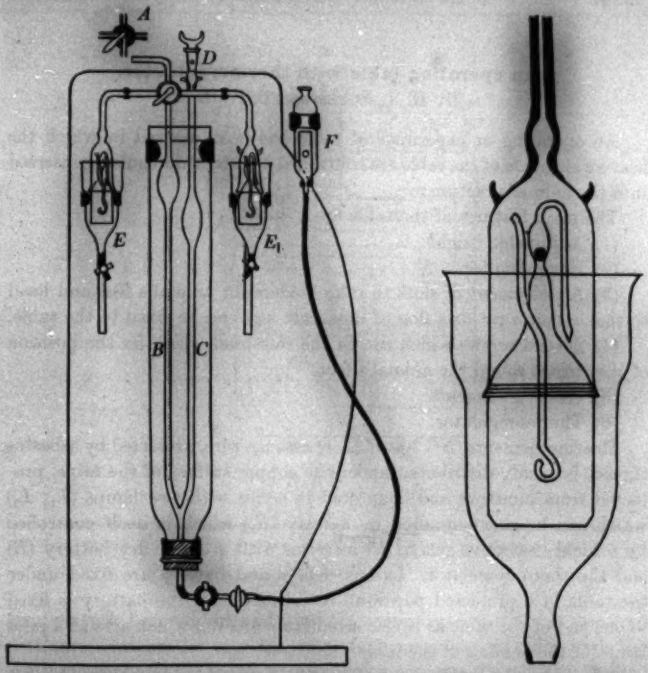
Modification of Haldane gas analysis apparatus. By H. J. Fuchs.
(*From the London Hospital Medical College*)

The system consists of a central stopcock *A*, as in D. T. Harris Haldane apparatus [1922], to which the usual 10 c.c. measuring burette *B* is attached. This is united at its lowest point to the thermobarometer tube *C*, the open end of which is free, simply finished off with a stopcock *D*. There is only one mark on the barometer tube, at the lower end, and this is at the same height as the 10 c.c. mark on the gasometric burette. Two absorption pipettes *E* and E_1 are placed on either side of the central stopcock on the upper end of the gasometric burette. These absorption pipettes make it possible to bubble the gas through the absorption fluid both when the gas is forced through it under pressure, and when it is

sucked back again, thus increasing the speed of absorption. Furthermore, these pipettes are easily removed, and cleaning difficulties are thus avoided; glass beakers complete this part of the apparatus. The fluid is covered by a thin layer of paraffin oil, and contamination by the atmosphere is therefore prevented.



Mercury is replaced by a saturated solution of Na_2SO_4-NaCl rendered slightly acid with H_2SO_4 and coloured with methyl red. This liquid does not dissolve any appreciable amount of gas [1932].

The levelling bulb *F* is moved up and down in an arc and remains in any position in which it is set.

This apparatus is as complete as the Haldane apparatus, and as efficient. It may be used with or without gas connexions between stop-

cock and absorption pipettes. Absorption itself is a far quicker process in this new instrument, and cleaning can be effected more easily and more quickly.

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An operating table with thermoregulator.

By H. J. Fuchs and C. Reid

An operating or experimental table has been devised in which the heating elements of the table are controlled by a thermoregulator inserted into the animal's rectum.

The main features of the table are:

(1) Adjustable height.

(2) Revolving top.

(3) Arrangement of slots to take holders for animal's feet and head so that either a rat or a dog of moderate size can be fixed to the table.

(4) Special screws which run in the slots and which fix the position of the strings round the animal's feet.

(5) Heating elements.

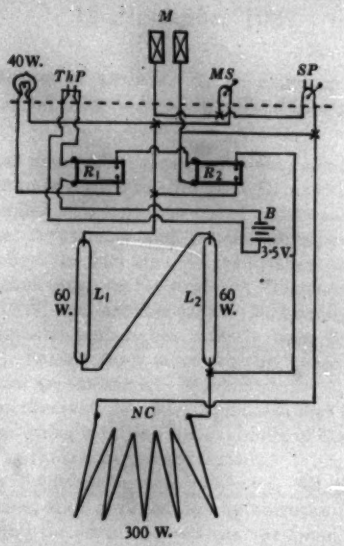
(6) Thermoregulator.

Heating elements (*NC* 300 W.)—resistance wire, protected by asbestos thread, is evenly distributed under the copper surface of the table, protected from moisture and connected in series with two lamps (L_1, L_2) which can be short-circuited by a relay (R_2) which is itself controlled by a highly sensitive relay (R_1) in series with a 3·5 V. dry battery (B) and the thermoregulator. Lamps, relays and battery are fixed under the table in a protected position. A voltmeter for the battery is fixed to one end of the table as is also a red lamp (40 W.) which acts as a pilot lamp for the heating of the table.

When the current is switched on, the resistance wire (*NC*), which is connected in series with the two lamps (L_1, L_2) (each 230 V., 60 W.), produces a small amount of heat. If this is lower than the temperature to which the thermoregulator in the animal's rectum has been set, the two lamps in series with the resistance wire are short-circuited, and the surface of the table warms up. When the temperature of the animal rises so that the mercury in the thermoregulator touches the contact points in its stem, the above short circuit is interrupted and the resistance

wire connected in series with the two lamps again so that the heat developed is lowered. Immediately the temperature of the animal falls below the "set" temperature the short circuit of the two lamps is re-established.

The thermoregulator is built on similar lines to the Beckmann thermometer, and the particular one supplied can be adjusted to any



temperature between 10 and 100° C., accurate to 0·1° C. It has two platinum contacts let into the stem for connecting in series with the 3·5 V. battery and the sensitive relay which actuates the relay controlling the heating elements of the table.

The advantages of the above thermoregulator are:

(1) The temperature of anæsthetized animals during operation or experiment can be kept constant within narrow limits at the normal for the animal or at another selected temperature.

(2) Repeated inspection of a rectal thermometer is obviated.

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Enzymic oxidation of amines. By H. Blaschko, D. Richter and H. Schlossmann. (*From the Physiological and Biochemical Laboratories, Cambridge*)

Extracts from mammalian tissue containing adrenaline oxidase also oxidized other amines related to adrenaline [Blaschko *et al.* 1937 *a*, *b*]. We have now examined a large number of amines and find that among others tyramine, tryptamine and certain aliphatic amines (e.g. iso-amylamine and heptylamine) are oxidized by these preparations.

An enzyme that catalyses the oxidative deamination of tyramine is known [Hare, 1928], and another enzyme that catalyses the analogous oxidation of aliphatic amines has recently been described [Pugh & Quastel, 1937]. The question arises whether these enzymes and the adrenaline oxidase are the same.

The following observations make it probable that there exists in fact only one oxidase which catalyses the oxidation of these different types of substrates. The three enzymes are similar:

(*a*) *In their behaviour towards inhibitors.* All three enzymes are cyanide-insensitive; they are inhibited by narcotics (e.g. octylalcohol). A specific inhibitor for adrenaline oxidase was found in ephedrine, and ephedrine also inhibits the two other amine oxidases.

(*b*) *In their distribution in animal tissues.* Substrates of the three different types are oxidized by extracts from liver, intestine, kidney, uterus, lungs and brain. Small amounts of the enzymes occur also in testicle and skeletal muscle (guinea-pig). Amine oxidases are also found in other vertebrates and in echinoderms (*Asterias rubens*, *Echinus esculentis*) and molluscs (*Patella vulgata*).

(*c*) *In that they show competition* and not addition when representative substrates of two of the enzymes are added simultaneously, and

(*d*) *In that they catalyse chemically similar reactions*, namely the oxidative scission of the C-N linkages. In the case of adrenaline, the reaction products are methylamine and an aldehyde [Richter, 1937].

The amine oxidase, as the system may be called, is distinct from the amino-acid oxidase and also from histaminase. Histamine is not oxidized by the enzyme.

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The pulmonary arterial pressure in the unanæsthetized dog.

By I. de Burgh Daly

The technique employed by London [1935] for the operation of angiostomy involves the transplantation of a piece of the omentum on a blood vessel previously painted with a dilute solution of iodine. Subsequently one end of a cannula is stitched to the artery and transplant, the other end being brought to the outer surface of the abdominal wall.

It has been found advisable to modify the method when the pulmonary artery is the selected vessel. Precautions must be taken to ensure the absence of pressure on the pulmonary artery during cardiac and respiratory cycles, and also to prevent the occurrence of pneumothorax. Dogs have been used.

The thorax is opened under general anæsthesia in the third or fourth left intercostal space and a slit 1–2 cm. long is made in the pericardium overlying the pulmonary artery. The wall of the artery is painted with a small quantity of dilute iodine solution, and one end of a flanged cannula is stitched to the cut edges of the pericardium with four No. 1 silk sutures by means of Jensen's curved needles. The outer coat of the artery is included in the sutures. The distal end of the cannula, also flanged, is brought through an additional incision made in one of the left intercostal spaces, and is then fixed underneath the skin by means of silver wire passing through holes bored in a rib and in the flange of the cannula. Alternatively, the cannula is brought through the dorsal musculature and fixed underneath the skin 4–6 cm. lateral to the dorsal midline by means of No. 3 silk which passes through a hole in the distal flange and round an adjacent rib. The alternative positions of the cannulæ are shown in the diagram which represents a cross-section of the thorax at the level of the trachea bifurcation. The skin margins over the distal flange are brought together and the main chest opening closed

so that the thorax is made airtight. A piece of omentum is not transplanted on to the pulmonary artery. Cannulæ have been introduced successfully into five animals.

Chromium-plated metal cannulæ 4 mm. internal and 5 mm. external bore and from 3–5 cm. in length were used in all operations except one, in which a cannula adjustable in length after insertion in the chest was employed to facilitate the accurate location of the central flange on the pulmonary artery.

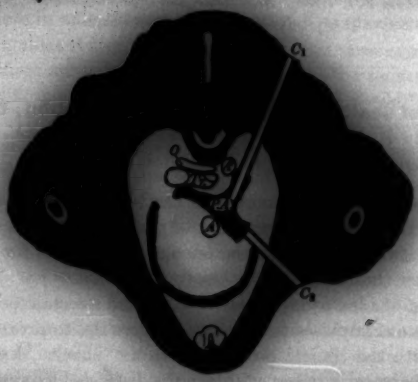

Fig. 1. A = ascending, A' = descending aorta. C_1, C_2 = alternative positions for cannula. O = œsophagus. $P.A.$ = pulmonary artery. $Tr.$ = bifurcation of the trachea.

Subsequent to the operation the mean pulmonary arterial pressure was recorded in three dogs by inserting a serum needle down the cannula into the lumen of the vessel. The skin was anæsthetized with novocaine. The needle was connected to a membrane manometer, clotting being prevented by filling the system with heparin solution or by Trendelenburg's [1924] method.

The resting mean pulmonary arterial pressure was found to range from 40 to 70 cm. H_2O. In one dog the resting pressure taken on the 62nd, 68th, 200th and 209th day after operation was 50–55, 45, 55 and 50–70 cm. H_2O respectively. These values are higher than those generally accepted [Dunn, 1919], and indicate a high degree of tone in the whole or in part of the pulmonary vascular bed. The pressure varies considerably if the animal becomes restless from standing or lying in one position for long periods of time; it may also be influenced by the presentation and taking of food.

Relation between size of testes and requirement of cortical extract in adrenalectomized drakes. By EDITH BÜLBRING

During the last 17 months when extracts of adrenal cortex have been tested on adrenalectomized drakes, it has been found that the sensitiveness of the birds to cortical deficiency changes. In spring much more extract is needed to keep operated birds alive than at other times of the year. The size of the testes at that time is also much greater.

Average weights of the testes have been determined for the different months, and it has been found that the weight of both testes in winter is about 3 g. In February the weight increases, it is already considerably more in April and reaches a maximum of 78 g. in May, after which there is a rapid decrease to the lowest figure in July.

When for each month the dose was calculated which kept adrenalectomized drakes alive for 16 hr., it was found that this dose was low in winter and was about 0·07 c.c. per hour. In April, however, as much as 0·3 c.c. per hour was needed, in May and June less, and in July 0·05 c.c. was usually sufficient. If these amounts of extract are plotted together with the weights of testes for different months, two parallel curves result. The weight curve of the testes reaches its highest point in May, whereas the dose curve has its maximum 1 month earlier. The figures are shown in Table I.

TABLE I

	Dose of cortical extract which kept adrenalectomized drakes alive for 16 hr. c.c. per hour	Weight of testes g.
January	0·080	6·9
February	0·090	12·0
March	0·200	30·4
April	0·300	63·6
May	0·180	78·0
June	0·170	29·0
July	0·050	0·6
August	0·070	2·2
September	0·060	4·2
October	0·065	3·3
November	0·075	3·4
December	0·070	2·8

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A differential viscometer.

By J. H. Shaxby

The apparatus consists of two horizontal capillary tubes *AB* and *CD* arranged in series, so that a liquid I fed in at one end *A* flows through the tube *AB* to a reservoir *BC* at the junction of the two tubes; from this reservoir a second liquid II is led through the second capillary *CD* to the final exit. The reservoir is completely filled with liquid and to it is attached a gauge of any kind suitable for measuring small changes of pressure. The initial pressure at *A* is kept positive and constant, the final pressure at *D* is atmospheric. Let P be the pressure at *A*, and p the pressure at *BC*, the pressure at *D* being zero.

With viscous flow the pressure to drive a given volume v in a given time through a given tube is proportional to v and to the viscosity of the liquid. Let subscript 1 refer to the tube *AB* and the liquid flowing to it, subscript 2 to *CD* and its liquid, which in practice is water, then

$$P-p=k_1\eta_1 v,$$
$$p=k_2\eta_2 v,$$

where the k's are constants depending on the length and bore of the tubes; v is of course the same for the two tubes.

Now replace liquid I by liquid II which now flows in both tubes, and keep pressure P constant,

$$P-p'=k_1\eta_2 v',$$
$$p'=k_2\eta_2 v',$$

where p', v' are the new values.

From these equations

$$\frac{\eta_1}{\eta_2}=\frac{P-p}{p}\,\frac{p'}{P-p'}.$$

I have been in correspondence with Dr London who informs me that he has now dispensed with the transplantation operation and has succeeded in placing cannulæ on to pulmonary arterial and venous branches. These cannulæ are brought to the outside of the thorax.

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The cause of death in acute intestinal obstruction; the nervous factor. By P. B. Ascroft and O. V. Lloyd Davies. (*Department of Physiology, Middlesex Hospital Medical School*)

Unrelieved complete obstruction of the bowel is always fatal: loss of fluid and electrolytes, and absorption of toxins, may alone cause death, but do not always appear to be enough to account for the typical symptoms, nor the fatal issue.

Distension of a hollow muscular tube beyond a certain point is known to set up nervous impulses that frequently give rise to severe pain, often associated with the clinical picture known as "shock", hence the suggestion that nervous impulses arising in the distended loops of obstructed bowel may be responsible for some of the serious symptoms of acute intestinal obstruction. Taylor *et al.* [1933] examined this theory and appear to have adduced strong confirmatory evidence.

These workers used dogs and proceeded as follows: "a rubber tube about ¼ in. in diameter, fastened to a sausage-shaped balloon, was placed in the [dog's] bowel, the continuity of which was not otherwise interfered with. The balloon was inflated until it caused a moderate distension of the bowel wall—60 to 110 mm. Hg. Most of the animals in which the pressure employed was around 100 mm. Hg. died within 24 hours with the usual symptoms and post-mortem findings of acute obstruction. The longest survival time was 54 hours." In these animals a barium mixture passed freely through the rubber tube into the bowel below, showing that there was no serious degree of obstruction. Control experiments were then done in the same way on denervated [deafferented] loops: "Twelve animals have been experimented upon in this way and none has shown the usual signs of obstruction. So far, however, we have been unable to keep any of these animals alive indefinitely with the balloon in position.

Two obstacles, insurmountable so far, have been encountered. In the first place, one or other end of the rubber tubing, which must be fairly resistant in order to remain patent against the pressure of the distending balloon, has eroded and perforated the bowel wall, usually in two or three days. For this reason peritonitis has been the cause of death in the majority of the animals in which the bowel was distended after denervation.... The second difficulty has been the movement downwards of the balloon and its passage per anum. This was the invariable termination of the experiment in animals which did not suffer perforation of the bowel. The highly significant fact, however, emerges from these experiments that not one of these animals died within 54 hours—the longest period survived by an animal with a distended, innervated bowel." The authors conclude, "...that distension of the bowel wall is the important factor in the production of the symptoms of experimental [acute] intestinal obstruction," and they affirm their belief that distension is important because it gives rise to nervous impulses arising in the distended bowel.

Because the disclosure of the extreme importance of nervous impulses in the syndrome of acute intestinal obstruction was so startling and because the control experiments on denervated loops were not entirely convincing, we performed some experiments designed to avoid the complications of peritonitis and intestinal obstruction. Cats were used. A loop of bowel 6–8 in. long was isolated and the continuity of the bowel restored. The isolated loop, with its mesentary, vessels and nerves intact, was then transplanted beneath the skin of the abdominal wall in a horseshoe-shaped loop, with each end forming an enterostomy on the skin. When the animals had completely recovered from the operation, a piece of thin walled rubber cigarette tubing was inserted through the loop, under nembutal anæsthesia and inflated with water at a pressure varying from 135 to 210 mm. Hg. The intestinal loop was thus tightly distended and remained so till the pressure was released, from 3 to 18 days later. Brisk peristalsis in the isolated loop was often evident. In no instance did an animal show signs of discomfort or deterioration in condition.

It is concluded that prolonged mechanical distension of an isolated transplanted loop of the cat's intestine with nerve supply intact does not produce the clinical signs of acute intestinal obstruction.

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Taylor, N. B., Weld, C. B. & Harrison, G. K. (1933). "Experimental Intestinal Obstruction." *Canad. Med. Ass.* XXIX, 227–236.

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where p', v' are the new values.

From these equations

$$\frac{\eta_1}{\eta_2}=\frac{P-p}{p}\,\frac{p'}{P-p'}.$$

If $\eta_1 = \eta_2 + \delta\eta$, where $\delta\eta$ is small, and $p' = p + \delta p$, where δp is thus the change recorded by the gauge when liquid II replaces liquid I in the tube *AB*,

$$\frac{\delta\eta}{\eta_2} = \frac{P-p}{p}\frac{p'}{P-p'} - 1,$$

which on reduction gives approximately

$$\frac{\delta\eta}{\eta_2} = \frac{P\delta p}{p'(P-p')}.$$

Now if k_1 is equal to k_2, i.e. if the tubes are of equal lengths and bores, p' equals $\frac{1}{2}P$, hence

$$\frac{\delta\eta}{\eta_2} = \frac{4\delta p}{P}.$$

It is of course impossible to ensure the precise equality of k_1 and k_2; it is easy to show that if they differ by as much as 1 p.c. the resulting error is 0·01 p.c. and is thus completely negligible.

In the first part of the experiment liquid I flows into the reservoir so that the liquid II entering tube *CD* will ultimately be mixed with a certain amount of I. If the reservoir is sufficiently large this mixing will not occur to an appreciable extent for a time much longer than that required for the pressure conditions to reach equilibrium and the reading of the gauge to be noted.

A simple adaptation photometer.

By J. H. Shaxby

This is a simple and easily constructed apparatus enabling measurements of the progress of dark adaptation to be recorded by a technique similar to that employed by Dr P. C. Jeans and his colleagues [1937] and by Dr Jeghers [1937] in their observations on the results of vitamin A deficiency.

The subject looks into a long box at the far end of which is a flashed opal glass screen illuminated from the other side by a lamp of suitable power. The screens can be completely darkened by an opaque sliding shutter.

The subject looks into the darkened box for 10 min., thus becoming dark adapted. At the end of the 10 min. the slide is removed so that light adaptation occurs. After 3 min. a second slide (opaque except for two small circular holes side by side near its centre) is slipped in and an Ilford

neutral wedge simultaneously introduced (with its length horizontal) by slots in the side of the box. The lightest part of the wedge should be set opposite the holes and then moved horizontally until the subject announces that he no longer sees light through the two holes. It is then moved back until the hole in front of the lighter part of the wedge is visible and the other invisible. The reading is noted and the opaque screen replaced. At the end of every 2 or 2½ min. a second reading is made, and the procedure repeated until a nearly uniform setting is obtained, indicating the completion of dark adaptation.

Graduations on the frame of the wedge indicate the wedge density at the point opposite the hole which is just not visible when a reading is taken.

The process of dark adaptation from a known degree of light adaptation is thus recorded; an illumination of about 1 foot-candle at the eye is convenient. Preliminary measurements about the middle and near the end of the preparatory 10 min. of dark adaptation are useful as checks on the later readings. It is important that the first reading, immediately after the cessation of light adaptation, should be made quickly as the change in the first half-minute or so of dark adaptation is very rapid.

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The hyperglycæmic response to transection of the mid-brain.

By J. M. Peterson and C. W. Startup

Whilst division of the brain stem of the cat at the level of the superior corpora quadrigemina and removal of the part above, under ether anæsthesia, cause the blood-sugar concentration to rise to a level of from 150 to 300 mg. per 100 c.c., this high level persists in only a small percentage of instances. The blood sugar is at its highest immediately after decerebration and usually falls to a normal level at a rate of about 25 mg. per 100 c.c. per hr. This fall is markedly delayed by prolongation of the ether anæsthesia, especially in animals with large stores of liver glycogen. Similarly, in the dog, decerebration through the superior corpora quadrigemina and below the pituitary body, results in only transitory hyperglycæmia in the majority of instances.

Asphyxia or intravenous injection of adrenaline causes the blood pressure and the blood-sugar level in the dog, decerebrated as described,

to rise to the same extent as in the dog anæsthetized with amytal or with chloralose. Variations, which are slight, occur in the hyperglycæmic response with variations in the initial blood-sugar level and in the glycogen content of the liver.

If we accept the view that the hyperglycæmic response to brain lesions is due mainly, if not solely, to secretion of adrenaline, it would appear that removal of the brain above the superior corpora quadrigemina causes this secretion mainly during the actual operation and that the response is intensified by ether anæsthesia. At the same time, the duration of the ether-decerebration hyperglycæmia may be more prolonged than one of similar height produced by a single intravenous injection of adrenaline either in the same decerebrate dog or in the dog anæsthetized with chloralose or with amytal.

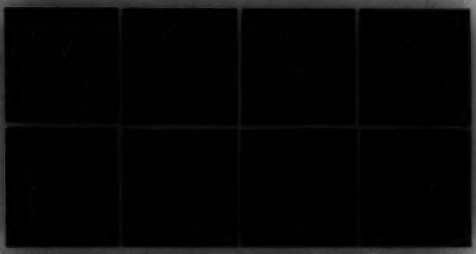
The hyperglycæmic effects of adrenaline and of asphyxia, which are probably both due to a direct action of the former on the liver, appear to be unaffected by either amytal or chloralose anæsthesia.

The expenses of this work were in part defrayed by a grant from the Medical Research Council.

Cannibalism and chemotaxis in the hen "monocytes" *in vitro*. By F. Jacoby. (*From the Department of Physiology, University of Birmingham*)

It is generally assumed that one mode in which the organism disposes of worn-out monocytes is by phagocytosis [see for instance Cowdry, 1934]. The following photographic observations, made on uniform populations of hen-blood monocytes *in vitro* (living in diluted serum), show that under certain conditions these cells are actually eaten by their fellows. This kind of "cannibalism" was observed in experiments in which the conditions for the cells had become less favourable owing to inadequacy or exhaustion of the food (diluted serum) supplied. The flask culture technique and the continuous photographic method used were the same as referred to in a previous communication [Jacoby, 1937]. On analysing the films these phagocytoses were discovered. In one experiment alone, lasting about 70 hr., 27 phagocytoses were encountered among a population of 260 cells in the field photographed ($\frac{1}{4}$ mm.^{2}). Apparently it is always only dying or dead cells which are phagocytosed. The course of events, as far as these films (6 min. interval between the pictures, 150× magnification) allow an analysis, is as follows: Before the cell

which is going to disintegrate[1] rounds off, it often passes first through a state in which it exhibits a crenated edge (Fig. 2). This might be due to an irregularity in the process of withdrawal of the pseudopodia. The smoothness with which this takes place during healthy life is already lost. Then, the cell rounds off (Fig. 3). This round state as it appears in the photographs of the living culture resembles very much the rounded-off phase in mitosis; but it differs from the latter, at least in these serum cultures, in two respects: (1) it is less refractive, and (2) the sphere formed is on the average smaller and tends to shrink further. The next occurrence is that the nearest neighbouring cells move straight towards the rounded cell thereby stretching their bodies so that they look like spokes radiating



Figs. 1–8. Eight successive photographs (paper negatives) of living hen-blood monocytes culture (8407 M 23) on the 29th day *in vitro*. ↓ Cell which is going to disintegrate. × Cell which has engulfed bulk of dead cell. Magnification about 120 ×.

from the dead cell as the centre (Figs. 4–6). This "star-figure" is a very characteristic feature of the process. By that time the "fore-ends" of the aggressors are lying closely together and are hiding the dead cell so that the actual process of engulfing could not be followed, quite apart from the low magnification used and the relatively long time intervals employed. Then, after some time the aggressors move apart (Figs. 7–8); the dead cell has disappeared, and in many instances it was possible to see that it had been enclosed as a round body within the cytoplasm of one of the aggressors (see × in Fig. 8). This was especially evident in cases where the dead cell contained many fat droplets whilst the others were transparent. After phagocytosis the engulfed complex of fat droplets could then be seen within one or more of the aggressors. Most of

[1] For shortness the disintegrating cell may be called "dead cell" and the phagocytosing cells "aggressors".

the dying cells, however, disintegrated right from a transparent state without passing through an intervening state of "fatty change" (Figs. 1–2).

The very sudden and straight moving of the aggressors towards the dead cell leaves no doubt that this takes place under the influence of a definite stimulus, that this movement is not a chance movement but is definitely directed, in short it reveals a positive taxis. A thigmotactic effect can be ruled out as in these cultures there is no coagulum which, if it were present, could convey a mechanical stimulus, a kind of stress, from the shrinking disintegrating cell to the surrounding cells. Thus, in all probability the stimulus which attracts these cells must be of a chemical nature, a substance or some substances released from the dying cell. These substances become quickly diluted as they diffuse out into the medium. Measurements show that the radius of effectiveness is about 25μ; i.e. cells wholly or partly within the circle of this radius will be attracted by the dying cell whilst those further away will not show any sign of oriented movement.

These observations, therefore, give strong evidence that cannibalism is one way by which the fate of monocytes is decided and that in this process a positive chemotaxis is involved.

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